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The Potential of Living Plants Harnessing Bio-Electricity through Bio-Photovoltaic Device

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ABSTRACT

The electricity crisis is determining the most pressing issues in the world that is escalating synchronously. Currently, a variety of sources are used to generates energy, but the by-products are extremely detrimental to peoples and the environment. In the present study, we developed and fabricated a low-cost and eco-friendly technology i.e. bio-photovoltaic device (BPVs) that uses an organic framework to produce green energy. Indeed, the BPVs device utilises the specific characteristics of the plant-microbes relationship in the rhizosphere region of the plant to modify solar energy into electrical energy through the bio-electrochemical method. The BPVs device was formulated in a grass *e*-table under the observed natural condition for the power yield of 0.57 ± 0.2V to 4.23 ± 0.2V at 30 days of the incubation period. The current study was pointed on harnessing bio-electricity using the biosystem which also addresses the existing electricity crisis. However, this technology is still undergoing many pieces of research work but has a good potential change worldwide and providing sustainable energy without harming the plants and environment.

Key-words: Bio-Photovoltaic device, Grass *e*-table, *Cynodon dactylon*, Bio-Electricity generation.

INTRODUCTION

In recent decades, the feasting of oil and gas has increased due to population growth, industrialization, and urbanization, leading to an energy calamity and environmental effluence [1]. Therefore, these are basic needs is fulfilled by paying high capital prices (Approx. 30 billion per year) an example for the economic survey of India. Biomass has attracted a lot of interest as

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a potential source of energy due to its abundance and flammability. However, an estimate of over 67% of electricity is formed of fossil fuels

than most of the researchers undergoing on harnessing electricity from renewable and sustainable sources like solar, wind, water, nuclear and biomass. In present time the world-wide electrical energy is obtained from nuclear reactors (13.4%), hydropower and wind (16.2%), solar and biomass (3.3%). However, there are some disadvantages to renewable energy sources [2]. These are the main source of electricity production by fossil fuels. The Bio-Photovoltaic device produces green electricity by using plants and mutual interaction of microbes that oxidized the organic residues harvest an electron. These electrons trapped by the conductive electrodes situated bio-system as well as the assimilation of the anode and cathode nearby the plants-roots and soil-surface causes the electrons to be fascinated towards the anode due to its negative charge and proton is involved towards cathode due to its positive charge thus generates green electricity through the bio-electrochemical system as depicted in Figure 1 [3]. This means that they alter chemical energy into electrical energy using biological material during photosynthesis. This is founded on the natural process without harming living plants and the environment. This innovation is a supportable and renewable process without zero discharge and obstruction for farmable land and nature. This is a good scope for the usage of BPVs is perfect in wetlands as a massive waterlogged spot is required [4].

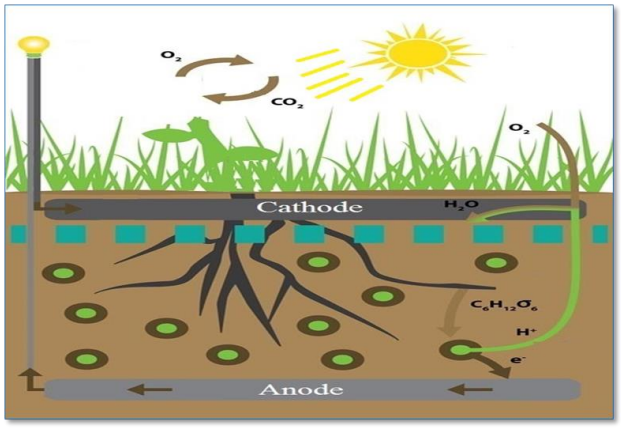

Fig. 1. Showing of Bio-Photovoltaic device (Bombelli *et al*., 2016) [3]

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MATERIALS AND METHODS

Collection of Plant Material

During the experimental work, the grass species of *Cynodon dactylon* was collected from the departmental garden, Rani-Durgavati University, Jabalpur (M.P.). The development of bio-photovoltaic device (BPVs) for the initial green electricity generation. The *Cynodon dactylon* is a plant genus classified under the Poaceae family which is usually grown in the soil salinity place that is recognized of Indian name called as Dooba Grass.

Experimental Setup of Bio-Photovoltaic Device

In this work, the bio-photovoltaic device was the fabrication of cathode and anode site and assembly was performed in an indoor laboratory condition. At BPVs was built by using cylindrical plastic pot which is commonly used in India for household plant growth. The BPVs setups consist of plastic container, with the dimensions of 8.5cm x 9cm x 8.5cm (length x width x height). The cathodic compartment comprises of copper electrode but proton exchange membrane (PEM) that was not used in the cathode chamber. Copper electrodes placed on the top of the soil surface. While other zinc electrode inserted near the plant-roots of *Cynodon dactylon*. Assembling the BPVs device, an electrical network to make a complete circuit using alligator clips and electrons move through in the complete circuits. The entire work was executed without connecting any resistance load. After 5-7 days of the proper maintains of growth and development of living plants, set of water was added and electricity potentials in BPVs device were recorded according to the method described by Smil *et al*., (2010) [5].

Influence of Light and Dark Condition

The power output was measured in voltage on an hourly basis from 10 AM to 5:30 PM at 10 days during light and shade condition. The average voltage obtained for each of the BPVs cells was intended and designed for BPVs performance. Similarly, in the same process for the output voltage was checked during the dark condition and measured for each of BPVs device for 10 days respectively the readings were recorded with the help of digital multimeter [6,7]

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To Formulation of Grass *e*-table for Bio-Electricity Generation by BPVs device

After the optimization of various condition for the maximum generation of green electricity than the formulation of grass *e*-table constructed was done. The couples of conductive electrodes were used as copper and zinc and the zinc placed in contact with the plant roots. While a set of copper electrode was submersed in the soil-surface. The copper cable was used to connect with both electrodes as the current collector. The performance of the Grass *e*-table device can efficiently be improved by connecting 12 BPVs devices and enhanced the power density, connected in the series arrangement of BPVs device and measurement of electricity with digital multimeter [8].

RESULTS AND DISCUSSION

The significant results found from the present examination are compiled under this work along with the results and discussions for the Bio-Photovoltaic device (BPVs) of potentials differences with light and dark conditions.

Influence of Light and Dark Condition

To scrutinize the effect of sunlight on the electric output, the BPVs pots were kept in light (L1) conditions in the replicates of three for 10 days. Annotations were recorded for both the conditions with the help of multimeter and that was found for the samples with and without plant. For L1 under light condition, the maximum generated potential of 0.60 ± 0.02V with plant and 0.33 ± 0.02 V without plant (control) was observed on to 10 days respectively. The set up of S1 were placed under shade condition measurement of voltage for BPVs pots. For S1 under shade condition, the maximum generated potential of 0.50 ± 0.02V with plant and 0.33 ± 0.02 V without plant (control) was observed on to 10 days respectively as presented in figure 2. Chlorophyll is necessary for the principle of photosynthesis and the release of electrons used in the production of electricity. Similarly, the green electricity production from *Epipremnum aureum* for the maximum voltage output 195mV was achieved 10 days of incubation period in P-

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MFCs setup. Furthermore, Pamintuan *et al*. (2020) harvested bioelectricity form three house plants like spider plant

(*Chlorophytum comosum*), Portulaca flower (*Portulaca oleracea*) and Dumb canes (*Dieffenbachia amoena*). In that setup of P-MFCs was obtained of 0.58 V attained per plant [8].

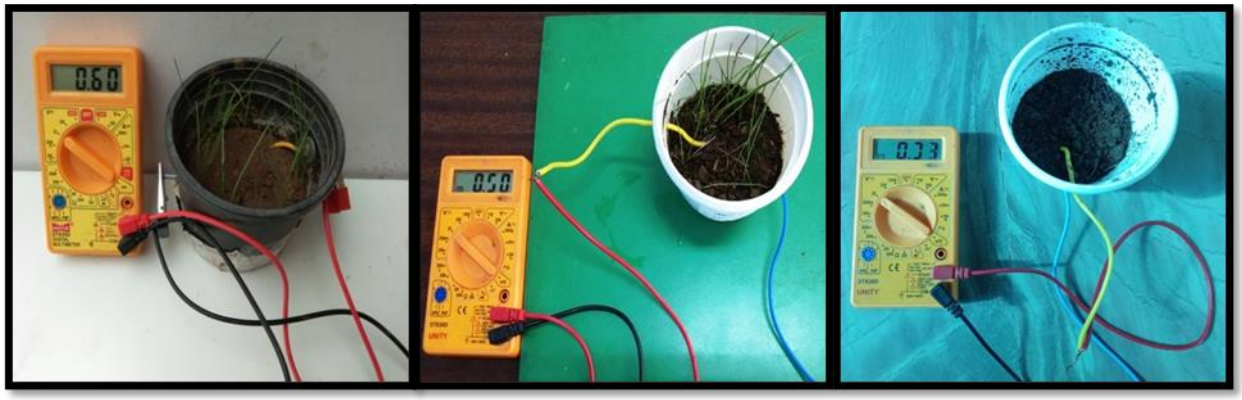

Fig. 2. BPVs pots generate in voltage with light (L1), shade (S1) and control Condition.

To Formulation of Grass *e*-table for Bio-Electricity Generation by BPVs device

This BPVs system-generated initially reading was low then after 7 days of incubation, then steadily raise the voltage. It showed the increasing trend up to 30 days and an identical well-maintained result with values was recorded 0.57 to 4.24 ± 2V for consecutive to study the effect of light on voltage and it was detected that voltage increased proportionally to the duration of sunlight. Finally, attained to evaluate of the effect of the grass *e*-table designed upon the generation of voltage using *Cynodon dactylon* in BPVs device was utilized as an energy source. It was found that electricity generates a maximum voltage of 4.24 ± 0.2 V on the 30 days of incubation as shown in figures 3 and 4.

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Fig. 3. Showing of bio-electricity generation in Grass *e-table*

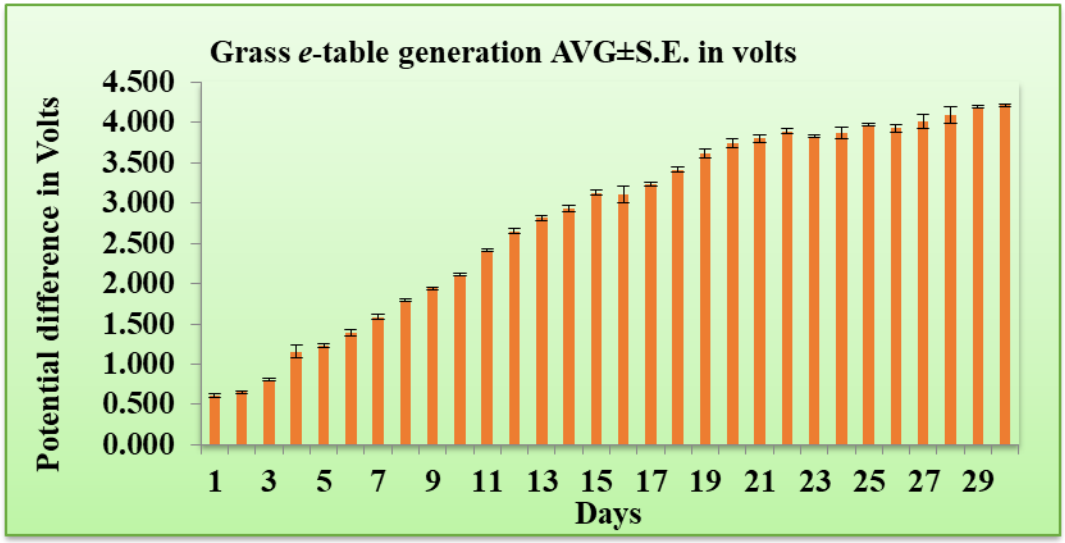

Fig. 4. Electricity generates in volts from grass e-table with standard errors bars

CONCLUSION

The current work has been shown the proof of principle constructed the biophotovoltaic device technology and signifies an innovative approach for using plant rhizodeposits that help as a substratum to bacteria for produce green electricity. The main emphasis of this study was on bio-electricity generation with the help of *Cynodon dactylon* by using bio-photovoltaic device.

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Further, formulate in grass *e*-table for enhanced production of green electricity it can be used at different places of the city where electricity consumption is high like in shopping malls, government office and corporate etc., in such designed ways that bring the city look like smart-city. This technology is eco-friendly and providing sustainable energy. This non-destructive method is cost-efficient and can be set up in rural as well as in urban areas (green roofs) to meet the rising demand for electricity and increase in the area as well as the number of grasses that would be put to use for the production of bioelectricity. The study also suggests fine-tuning technology to make better use of wastes for commercial purposes, such as the generation of alternative energy, to fulfil the demand in the world.

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CONFLICTS OF INTERESTS

There is no closing conflict of interest concerning the current research. Authors have to examine and announce the manuscript and agreed to submit it for publication.

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SUCCESSFUL REINTRODUCTION OF CAPTIVE CHITAL *(AXIS AXIS)* IN WILD HABITAT

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Abstract

Chital is medium sized deer and an important prey base for top carnivores. Present Study area includes Kota Zoo (Captivity) and Mukandra Hills Tiger Reserve (Wild habitat) which was notified as Tiger Reserve in 2013. Chital from captive areas including Kota Zoo were translocated to Mukandra before tiger reintroduction to increase prey base. In spite of significant difference in behaviour of captive and wild Chital, translocated animals were successfully reintroduced and able to survive in natural habitat in Mukandra. Chital was adapted very quickly to natural condition and even extreme conditions in wild. Vigilance behaviour which was rarely observed in captivity in absence of predators is of utmost importance for survival in wild from predation and as observed vigilance behaviour was increased surprisingly after reintroduction, indicated that vigilance is an inborn behaviour in Chital. Chital was also able to survive in reduced physical condition during pinch period in wild. Reintroduced Captive Chital population spent more time in feeding in wild as compared to already existed wild Chital population. This might be probably because of less adaption of tanslocated Chital population for feeding natural vegetation in wild habitat, specially feeding on fallen leaves during winter season so they spent more time in search of food. Hence captive Chital from various zoos and biological parks of India was successfully reintroduced in the new natural wild habitat of Mukandra.

Key words: Prey, Behaviour, Vigilance, Feeding

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Introduction

India has been a biodiversity rich country. Biodiversity is crucial for balancing the ecosystem. Studies on predator's diet revealed Chital as most abundant key prey species for big cats and it constitutes a bulk of Tiger's diet (Johansingh, 1992; Khan, 1995; Wegge et. al., 2009; Khan et. al, 1996; Stoen and Wegge 1996; Bagchi, et. al., 2003; Biswas and Sankar, 2002). Thus, management and conservation of Chital is essential for survival of Tiger in Tiger reserves. Tiger Reserve and Zoos plays vital role for wild animal conservation and management. Zoo also plays significant role in conservation of wild species and now a day's Zoos are converted in to Biological Parks which ensures to provide natural environment to wild animals to maintain their wild behavior necessary to survive when reintroduced in wild. The systematic acquisition and disposal of Chital from Zoo play key role in their management and conservation. The disposal of surplus animals from captivity to wild is done to fulfill requirement of wild habitat. Chital in captivity also provides important gene pool for future perspectives. Zoo play a role for present and future scientific management. This is executed through captive breeding and rehabilitation in wild.

Chital is native of India, Bangladesh, Nepal and Sri Lanka and introduced in Australia and United States of America (Prater, 1934; Schaller, 1967). Chital prefer ecotone between grasslands and dense forest at lower elevation of dry and mixed deciduous forest (Moe and Wegge, 1997). Chital usually avoid the interior of the forest and aggregate along road side clear areas in woodland and grassland habitats (Noor et. al., 2013; Sharma and Sharma, 2014; Varman and Sukumar, 1995). Chital favours ecotone between the forest and the grassland, they do not like closed dense forest (Chandra, 2013). Chital remain associated with langur in wild (Newton, 1989). Chital (*Axis axis*) feeds on a number of plant species as food (Johansingh and Sankar, 1991) and grazing to browsing ratio changes in different season considerably which depends on the availability of food (Khan, 1994).

Study area includes wild habitat Mukandara Hills Tiger Reserve (MHTR) and captivity Kota Zoo (KZ). Mukandra hills Tiger Reserve was established as National Park in 2012 and as Tiger Reserve in 2013. It was a Tiger Reserve without tiger in 2013 and since then main focus was on

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tiger reintroduction in MHTR. For tiger reintroduction prey base was an important issue. Chital was the most abundant ungulate prey species among all ungulate species in MHTR (Khan and Sultana, 2014).

Methodology

Observational Research Design was used in the present study conducted during 2017-18. Direct observation is the best method for behavioural study of animal in field. Behavioural observations was done by scan sampling techniques (Altmann, 1974). The preferred method for identification of animal in wild is to observe the animals with binoculars (Mathur, 2002). Data were collected mainly from primary sources and a little from secondary sources as well. The primary data were collected from direct observation of study animal. Secondary data were collected regarding Chital translocated in wild habitat MHTR from captivity from DCF office (wildlife), Kota and DCF office, MHTR to observe the reintroduced Chital population.

The Chital herds were continuously followed as long as possible on foot or on Motor-cycle from dawn to dusk in different time intervals. Observations were recorded at the adequate distance from the animals so as to avoid the influence of observer's presence on the natural activity of Chital herd. The field observations were carried out with the aid of binocular and Camera. The data were recorded on elaborate check sheets (Ethograms).

Results

The study was conducted in Kollipura and Borabas Range. Chital population was consistently increasing since the declaration of Mukandra National Park in 2012 and Mukandra Hills Tiger Reserve in 2013. Reintroduction of Chital during the study period in MHTR was done from captivity Kota Zoo and other Captive habitats such as Jaipur Zoo, Shahpura Sanjay Van, Ashok Vihar Deer Park. Similarly. Chital were also translocated from Jodhpur zoo and Rashtrapati Bhawan, New Delhi to MHTR and attached Bhainsrodgarh rehabilitation center. Tranlocated Chital preferred to live in the vicinity of road sides initially after translocation. Later they adapted quickly to wild habitat and exhibited the alert behaviour in increased frequency. It was seemed to be positive sign for tanslocated Chital population to survive from predators and any

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other danger in wild. Health condition was better in wild (MHTR) as compared to captive habitat (KZ) might be due to availability of different type of food plants, browse, fallen leaves and fruits rich in nutrients in different season. Chital population was seen to increase rapidly in new introduced habitat.

Discussion

Prey population decides the predator population hence conservation and reintroduction planning for top predators like tiger, lion and leopard in human dominated fragmented area (shared with livestock) requires information on ecology and ethology of prey population (Bagchi et. al., 2004; Nama et. al., 2013). Chital was the most abundant ungulate key prey species in present study as was discussed in earlier studies (Schaller, 1967; Eisenberg and Lockhart, 1972; Johansingh, 1983; Dinerstein, 1980). The larger groups were composed of many basic family units, which usually observed united together temporarily during feeding, travelling and resting (Graf and Nicholas, 1966).

Group size and Social organization of group played an important role in behavioural patterns displayed by animal. Chital was observed to live in small to medium sized herds same as earlier studies (Prater, 1971; Nikica, et. al, 2008). Among all wild ungulates, Chital showed highest mean and typical group size (Bagchi et. al., 2008; Dave, 2008). They were used to aggregate from evening onwards as the scattered deer herds for grazing move towards their night resting places in open grassy area similar to previous studies (Sharatchandra and Gadgil, 1975).

Chital group members were not associated with each other by social bonds and frequently leave or join a group indicated fluid nature of their herd. Group size was determined by availability of food, habitat structure, predation pressure and group composition ((Barrette, 1991; Khan and Vohra, 1992). Mean group size changed considerably between seasons depending on the availability of food. The herd size was observed maximum during wet season due to increased availability of forage in monsoon as in previous studies (Ramesh et. al., 2012). The fission fusion pattern of grouping in Chital is thus affected by many factors and social behaviour was also involved to explain the changes in group size (Raman, 1997).

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Mixed herd was the basic unit of the social organization in Chital whereas female-young and all male associations were temporary associations (Tak and Lamba, 1984). Fawn sex ratio represented an equal sex ratio at birth and an approximate equal sex ratio is to be expected in an area which is devoid of selective predation (De and Spillet, 1966). Chital Adult sex ratio and population structure was biased towards female (De Silva and De Silva, 2001; Karanth and Sunquist, 1992; Majumder, et. at., 2013). Chital Adult sex ratio was changed seasonally because more stags joined the groups during breeding season (Srinivasulu, 2001).

In Chital, males with all type of antlered condition were present which indicated the fact that breeding occurs throughout the year. This fact was further proved by the occurrence of fawning throughout the year. Almost all fertile females conceived and give birth to young. The ability to exist in a reduced physical condition, that Chital has shown was of obvious survival value to any animal facing too much kind of adverse (Krishnan, 1972). Fawning was also associated with velvet antlered condition (Mishra, 1982). Thus, almost all females of Chital were successful in reproduction while only few dominant males were involved in breeding activities (Sharatchandra and Gadgil, 1980). Chital was a timid and social animal. It's antipredatory behavior contributed a lot to its social behavior (English, 1992).

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An Investigative Study on Growth of Light of AlGaAs/GaAs in Nanotechnological Life Sciences

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Abstract

In this exploring type research letter an investigative study on growth of modal confinement light gain of AlGaAs/GaAs type ternary nanomaterial in nanotechnological life sciences has been done by computational type nanotechniques under the number of NGILs (Nano Graded Index Layers). This type innovatively research letter provides a substantial contribution in nano type biological sciences because of their unique utilities. The spectral performances of growth of modal type light gain with energies of light photon in eV of AlGaAs/GaAs has been calculated and computed by spectral performances. In these spectral performances the peaks of spectra has been achieved at energy of photons ~1.5 eV correspondence wavelengths of light photons ~830 nm have been illustrated by appropriate graphical curves under various types of NGILs. Next the modal type behaviours of transparency energies of light photon of with different types of NGILs for proposed nano structure have been calculated by graphical results. Next, these types light of wavelengths ~ 830 nm have mostly been utilised in the optimization of a proper combination of higher penetrating abilities and cellular type interactions. Hence this wavelength's light source has also been used in the treatment of various sensitive type skin diseases in the nanotechnological biosciences and medical sciences.

Keywords: Growth of modal type light gain, Transparent type photon energy, Photon wavelength, NGILs, AlGaAs, GaAs

Introduction

The importance of heterogeneous type nanostructure in the nanotechnological sciences has been found to increasing order in recent time. In present time under the nanoscale type technological

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and engineering sciences the performances of heterogeneous type structures are very critical because of their unique optical light properties. The various types of experimental and theoretical research based innovative work in the all over world

have been done by the researchers. In several fields like medical science, industries, radar system, aerospace, photovoltaic and detectors areas, lasing type devices etc., the nanoscale type heterogeneous structures provide great role due to their several optical performances. The several types of optical properties of various nano scale type heterogeneous structures [1-5 and 17-18] have been investigated by the researchers. In general the heterogeneous type structures are formed by the process of combination of multiple hetero type junctions. The hetero type junctions are those junctions that are formed by interface between the dissimilar band gap nano materials. Among various nanoscale hetero type structures, the AlGaAs/GaAs nanotechnological materials have been very popular due to emission of radiations of ~ 830 nm wavelength. These type wavelengths have been of highly concern due to their potential performances in the fibre optic appliances based telecommunications due to diminish attenuation. These nanotechnological materials have been set up some additional reward such as gain stability at higher temperature, improved line width enhancement factor and photonic wavelength. The materials AlGaAs and AlGaInAs/InP [12-14] have also been reported as a platform on which the nanotechnological devices can be fabricated. For examples, the electrical results such as the I-V and C-V curves of the Schottky type diodes, which were fabricated on AlGaAs/GaAs nanotechnological materials, have been studied under the variations of barrier heights. In this paper an investigative study on growth of modal confinement light gain of proposed nanostructure in nanotechnological life sciences has been done by computational type nanotechniques under the number of NGILs. This research letter provides a substantial contribution in nano type biological sciences because of their unique utilities. The spectral performances of growth of modal type light gain with energies of light photon in eV of proposed nanostructure has been calculated and computed by spectral performances. In these spectral performances the peaks of spectra has been achieved at energy of photons ~1.5 eV correspondence wavelengths of light photons ~830 nm have been illustrated by appropriate graphical curves under various types of NGILs. Next the modal type behaviours of

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transparency energies of light photon of with different types of NGILs for proposed nanostructure have been calculated by graphical results. Next, these types light of wavelengths ~ 830 nm have mostly been utilised in the optimization of a proper combination of higher penetrating abilities and cellular type various interactions.

Computation and Theoretical Details

It has been clear that, now a day in the nanotechnological electronics and optical communication the yielding of optical gain [9-10, 15-16] has substantial role due its unique light properties. Commonly, the profit in optical light is given by yielding of light per unit original light intensity and per unit length of active region of structure. In the nano type optoelectronics optical type light yielding has been achieved when transitions of upward type are enhanced than transitions of downward type while in the equal condition of upward and downward transition the achieved yielding is negligible this type condition is termed as condition of transparent. When the power of absorption is higher than stimulated type emission then loss is obtained in the optical type light. An expression related with yielding of light gain as function of temperature and energy is give as below relation. This yielding gain equation is given by the reference [6].

$$G(E) = \frac{e^2 h}{2nEm_0^2 \varepsilon_0 c}\left[1 - \exp\left(\frac{E - \Delta f}{k_b T}\right)\right]$$
$$\times \sum_{nc,nv} \frac{|M_b|^2 f_c f_v}{4\pi^2 L_W} \times \frac{(h/2\pi\tau)dk_x dk_y}{\pi(\{E_{nc} + E_{nv} + E_{sg}\} - E)^2 + (h/2\pi\tau)^2}$$

The modal type confinement parameter has critical role in the computing of yielding of modal confinement light gain per cm in the nanotechnological optical communications. The fundamental equation of modal type confinement parameter is given as following type relations.

$$\Gamma = \frac{\int_{-W/2}^{W/2} |\varepsilon(z)|^2 dz}{\int_{-\infty}^{\infty} |\varepsilon(z)|^2 dz}$$

The rate at which light gain varies with respect to carriers per unit volume is termed as differential type light gain. The equation of differential type gain is expressed as below relation.

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$$G^{'}(E)=\frac{dG(E)}{dN}=\frac{8\pi^{2}m_{r}E}{c\varepsilon h^{3}L_{w}}$$
$$\times\int_{E^{'}}^{\infty}|M_{b}|^{2}\left(\frac{df_{c}(E)}{dN}-\frac{df_{v}(E)}{dN}\right)L(E^{'})dE^{'}$$

The rate at which index of refraction has been changed with respect to carriers is called differential type index of refraction is shown by below equation.

$$n^{'}(E)=\frac{dn(E)}{dN}=\frac{4\pi^{2}m_{r}E\lambda\tau}{c\varepsilon h^{4}L_{w}}$$
$$\times\int_{E^{'}}^{\infty}|M_{b}|^{2}\left(\frac{df_{c}(E)}{dN}-\frac{df_{v}(E)}{dN}\right)(E^{'}-E)L(E^{'})dE^{'}$$

The parameter of antiguiding is expressed in terms of differential type index of refraction and differential type gain by below equation.

$$G^{'}=\frac{dG}{dN}=\frac{4\pi}{\lambda}\times\left(\frac{1}{\alpha}\right)\times\left(-\frac{dn}{dN}\right)$$

The equation of relaxation oscillation type frequency is exhibited by following equation. In this equation, the brief details of appropriate terms can be exhibited in refs [7, 8 and 11].

$$f_{r}=\frac{1}{2\pi}\times\left(\frac{(cP)}{(n\tau_{p})}\times\{G^{'}(E,N)\}\right)^{1/2}$$

The expression of threshold type current is given as below relation.

$$I_{th}=\left(\frac{nJ_{0}WL}{\eta}\right)\exp\left[\left(\frac{1}{n\Gamma G_{0}(J)}\right)\times\left(\alpha_{i}+\frac{1}{2L}\ln\frac{1}{R_{1}R_{2}}\right)-1\right]$$

Computational Results and Discussions

Basically, light increments are the net amount of the stimulated type emission that a photon generates as it travelled in given appropriate distance. In the hetero type nanostructures, the light amplification is caused by photon induced transition of electrons from the c-band to the v-band.

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If the rate of downward transitions exceeds the rate of upward transitions, there will be a net generation of photons and enhancement or profit in optical type gain [9, 10] can be achieved. The modal type gain enhancement per cm versus photonic wavelengths for various NGILs and peak modal gain enhancement in intensity of light per cm versus number of NGI-Layers of nanomaterial AlGaAs/GaAs type heterogeneous structure under the various number of NGILs have been illustrated by left y-axis and bottom x-axis; and right y-axis and top x-axis, respectively in fig1. The value of peak modal gain enhancement tends to higher value as reduce in number of

NGILs due to increase in value of parameter of modal confinement. The highest value of enhancement in intensity of modal type gain per cm is achieved at the wavelength of 830 nm. This range of wavelength of light has critical importance in the utilisation of NIR applications to achieve the combination of higher penetration power and cellular interaction performances without any type absorption losses. The achieved modal type gain results correspondence to maximum modal type light gain of wavelengths (~830 nm) for lasing phenomenon have an essential contribution in current days for the applications of EM radiations as well as this type wavelength range has been also useful in fibre optic telecommunications by the method of TIR with diminished losses and attenuations in dB/km of light signals. Moreover, the emitted light of 830 nm wavelength range has been used in the treatment of skin type deceases and this range also provides the contribution in the determination of correlation between higher value of penetration power and interaction of cellular in the medical sciences in daily life applications.

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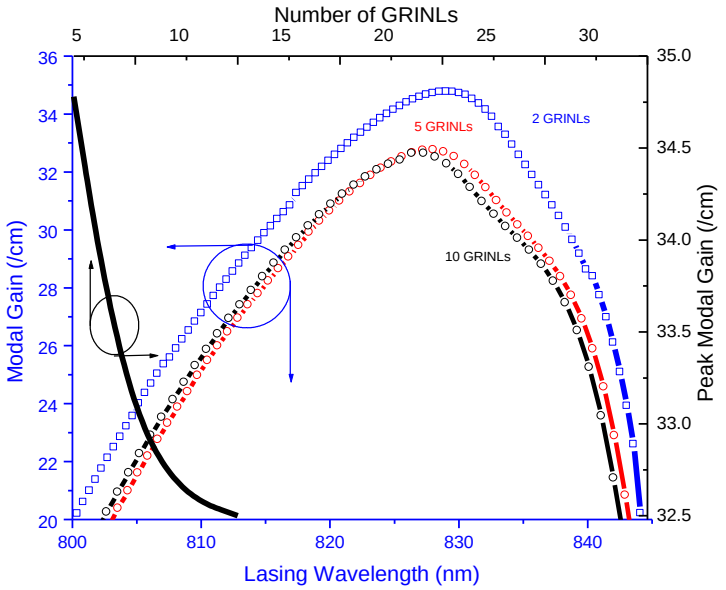

Figure1. Modal type gain intensity with photonic wavelength and peak modal type gain intensity with various NGILs for AlGaAs/GaAs

The compression in peak gain with various numbers of NGILs by left-y axis and bottom y- axis, and range of parameter of anti-guiding versus peak change in index of refraction by right y-axis and top x-axis for AlGaAs/GaAs type heterogeneous structure are shown

in fig 2. It has been cleared by fig 2 that the performances of peak type gain compression values have been reduced as increase in the number of graded refractive index nano layers i.e. the performances of peak type gain compression has reciprocal behaviour with the number of graded refractive index nano layers. And range of antiguiding parameter has also been reduced as enhancement in peak change in index of refraction i.e. range of auntiguiding parameter provides reciprocal performances with peak change in index of refraction. Hence, this types wavelength ~ 830 nm has been utilised in the optimization of a proper combination of higher penetrating abilities and cellular type interactions. Hence this wavelength's light source has also been used in

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the treatment of various sensitive type skin diseases in the nanotechnological biosciences, medical sciences and life sciences.

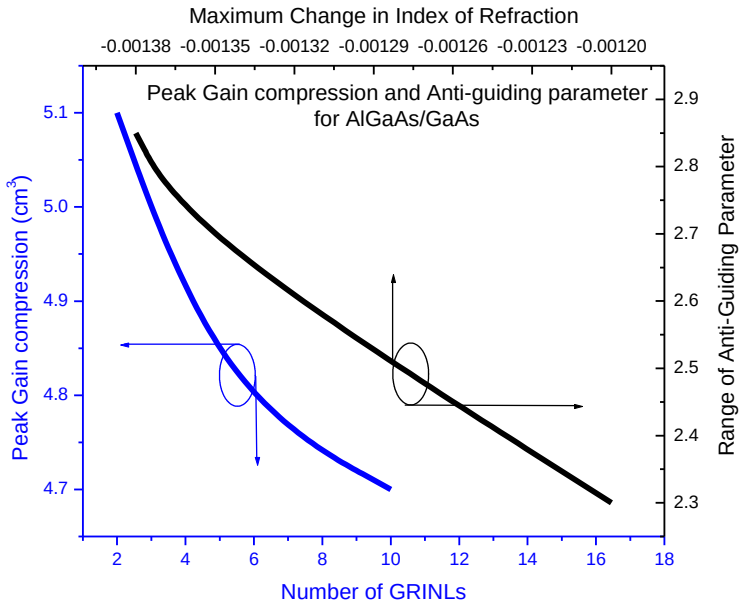

Figure 2. Compressional behaviour in peak gain with various numbers of NGILs and range of parameter of anti-guiding with peak change in index of refraction for AlGaAs/GaAs

Conclusions

Under the number of NGILs (Nano Graded Index Layers), in nanotechnological life sciences an investigative study on growth of modal confinement light gain of AlGaAs/GaAs type nanostructure has been done by computational type nanotechniques. This type innovatively research letter provides substantial contribution innano type biological sciences because of their unique utilities. The spectral performances of growth of modal type light gain with energies of light photon in eV of AlGaAs/GaAs has been calculated and computed by spectral performances. In these spectral performances the peaks of spectra has been achieved at energy of photons ~1.5

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eV correspondence wavelengths of light photons ~830 nm have been illustrated by appropriate graphical curves under various types of NGILs. The modal type behaviours of transparency energies of light photon of with different types of NGILs for proposed nano structure have been calculated by graphical results. These types light of wavelengths ~ 830 nm have mostly been utilised in the optimization of a proper combination of higher penetrating abilities and cellular type interactions. This wavelength's light source has also been used in the treatment of various sensitive type skin diseases in medical sciences and nanotechnological biosciences.

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To study the mode of attachment of monogenean fish parasite of the genus Bychowskyella Archmerow , 1952 in the fishes of river Gomti

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Abstract

The aim of this study was to investigate the mode of attachment of the genus Bychowskyella Archmerow , 1952 on the gills of the fish host . The host is collected from river Gomti Lucknow , India . It was found that parasite attach itself on the gills at the distal end and attach it itself in between two gill lamellae by its special rhomboidal haptor. An effect has been also done to study mechanical injuries to the fish. Studies were also carried out with the help of light and scanning electron microscope to study the impact of this parasite on the gills of host.

Keywords: Monogenea, haptor, gills, hamuli, gill lamellae

INTRODUCTION

The study shows that earlier monogeneans of the genus Bychowskyella attached themselves in between two secondary gill lamellae (Fig. 1 & 4) of the host. Maximum number of parasites was found on the fourth gill arch and their number decreased successively from fourth to first gill arch. They attach themselves more towards the distal border of primary gill lamella. (Fig 3)

Haptor (attachment organ) is rhomboidal (Fig3).it bears two pairs of hamuli, two connective bars, onchium, sclerotised rod, and 14 marginal hooklets of dissimilar shape and size. Each dorsal hamulus is without roots, stout base, long straight shaft, with a short recurved point and is dorsally oriented (Fig 8).

Dorsal connecting bar is straight, fenestrated in the middle, its ends articulate with the stout base of hamulus of its side. Ventral bar is paired, with long transverse shafts, both articulate in the middle and its free ends articulate with the base of ventral hamuli.

Bychowskyella Achmerow, 1952 as in earlier monogeneans, worms of the genus Bychowskyella attach themselves in between two secondary gill lamellae (Figs. 1 & 4) of the host. Maximum number of parasites were found on the fourth gill arch and their number decreased successively

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from fourth to first gill arch. They attach themselves more towards the distal border of primary gill lamella (Fig. 3).Haptor is rhomboidal (Fig. 3). It bears two pairs of hamuli, two connective bars, onchium, sclerotised rod and14 marginal hooklets of dissimilar shape and size. Each dorsal hamulus is without roots, stout base, long straight shaft, with a short recurved point and is dorsally oriented(Fig. 8). Dorsal connecting bar is straight, fenestrated in the middle, its ends articulate with the stout base of hamulus of its side. Ventral bar is paired, with long transverse shafts, both articulate in the middle and its free end sarticulate with the base of ventral hamuli. The narrow end of onchium passes through the fenestrated part of dorsal bar and articulates with the sclerotised rod, which on the other end supports and helps in the movement of ventral bar. Ends of ventral bar are articulated with the base of ventral hamulus present on its side. When the sclerotised rod and onchium move upwards, the patches on the dorsal hamuli are pushed downward, thus moving dorsal hamuli towards each other, with their points directing outwardly. Movement of sclerotised rod makes the paired upward The bar to form an inverted V-shaped structure. The ventral hamuli, attached to free ends of ventral bar, are ventral towards each other, with their points directed pushed. The parasite therefore detach from outwardly its site of by retracting its dorsal as well as ventral attachment. hamuli

Now, the downward movement of linked onchium and rod pulls the patches upward moving dorsal Sclerotised hamuli apart from each other, with their points outwardly. The simultaneous movement of sclerotised rod makes the ventral bar to go straight or somewhat curved paired upwardly. By this movement, ventral hamuli are pulled apart from each other and their points outwardly. Thus, the parasites attach to gill tissue (Figs.2,3,6 & 7). The two movements, towards and apart of dorsal and ventral hamuli detach the parasite and attach itself respectively. Points of dorsal and ventral hamuli are directed opposite to each other so the dorsal hamuli are oriented dorsally and the ventral hamuli are oriented ventrally from the centre of the disc. Thus, the parasites attach in between two adjacent secondary gill lamellae.

Materials and Methods:

The parasites is collected from fresh water river Gomti district Lucknow , with the help of fisherman by using some nets . Host were brought to the lab and identified with the help of the

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fish base (Froese & Pauly, 2018) . Gills were kept and removed in petri dishes containing water . Mode of attachment and locomotion of live worms were observed under Stereomicroscope (Leka EZ4HD) worms were fixed in 3% formalin . method of staining mounting and illustrating were those of Agrawal at (2016). Identification of parasite is done with the help of an encyclopedia od Indian Monogenoidea (Pandey and Agrawal, 2008) by using Olympus BX50 phase contrast microscope Tokyo Japan.

For Sem studies, live parasite were fixed in 25% gluteraldehyde (for 6h at 4 degree C) washed with 0.01 mole phosphate buffer (ph 7.4, 20 min x 3) and post fixed with 1% aqueous osmium tetoxide for 3hr . Subsequently they were washed with distilled water and dehydrate in the series of ascending grades of ethanol and dried by critical point drying method specimen were mounted on the aluminum stubs , coated with gold palladium (15mm thickness) and examined microscope (LEO,430, UK) Photomicrograph were take for further studies.

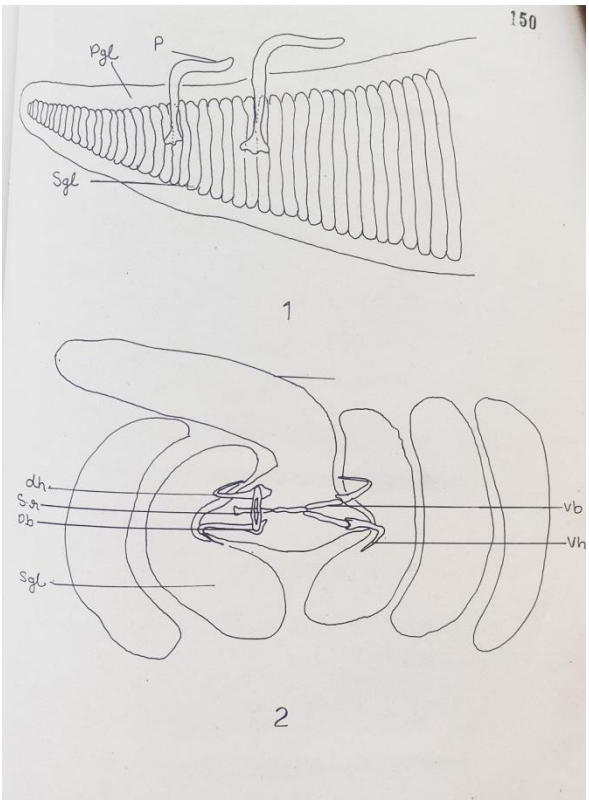

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Fig:1 parasite attached between secondary gill lamellae.Fig: 2 Haptor showing , Orientation of hamuli and connective bar.

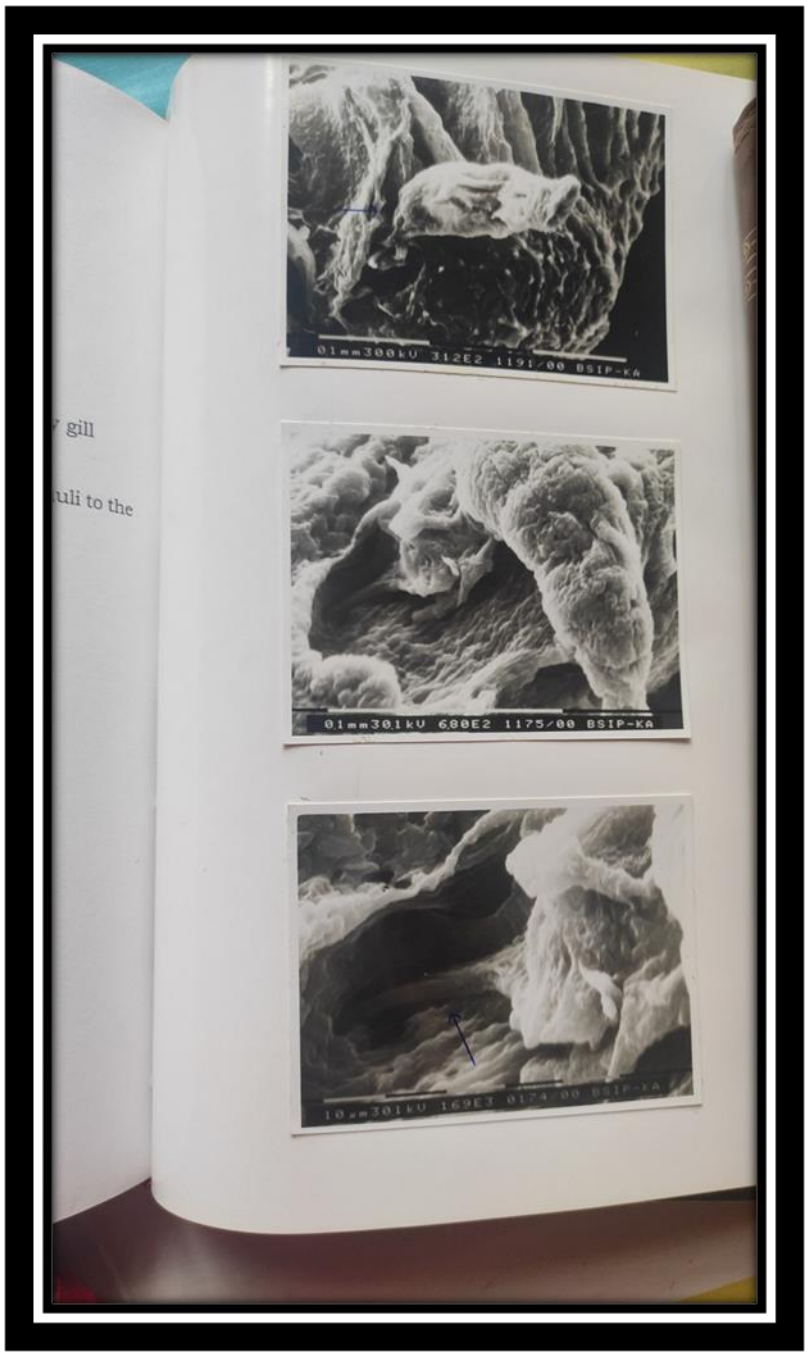

Fig: 6 Parasite attaching in between two secondary gill lamellae (SEM)

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Fig: 7 Orientation and penetration of the dorsal hamuli to the gill tissue (SEM)

Fig.8 Enlarged view of the same.

LOCOMOTION

Bychowskyella, moves on secondary gill lamellae but the movement is quite fast. During locomotion, it was that the worm site stretches (Fig. 10) (, Fig.9 the) secondary its body and observed for attaching anterior region at the site of attachment gill searches. It puts its lamellae (Fig. 11) and detaches its haptoral region from the previous site (Fig. 12). sbsequently, the parasite puts its haptoral the attached anterior portion. The worm near region itself to the other site in the inter lamellar spaces of adheres gill lamella from the previous the secondary one (Fig.13). , the haptoral region is retracted and is Subsequently with the help of its adhesive apparatus. Then attached its body, it again searches for some other site of waving and bends its body towards the proximal end attachment of primary gill lamella.

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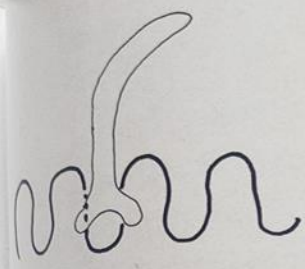

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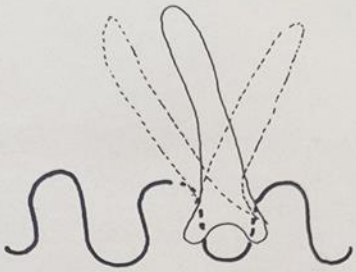

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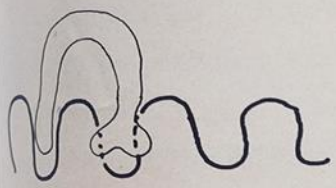

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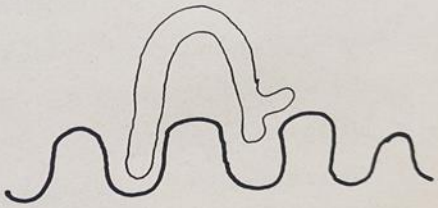

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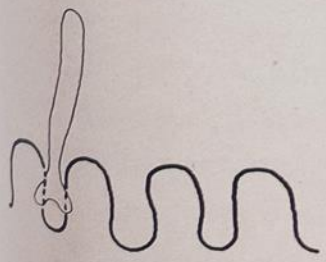

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DISTRIBUTION

The number of parasites are more on the anterior part of fourth gill arch. The number of parasites were least recorded on the IInd gill arch, on both anterior as well as posterior regions of hemibranch. The parasite attaches lamellae. The medial section of the 4th gill arch was the favoured site of attachment of Bychowskyella. in the interlamellar spaces on the secondary gill.

DISCUSSION

The adhesive attitude of Bychowskyella differs from other monogenean parasites, the haptor is rhombiodial and it bears two pairs of hamui, two connecting bars,onchium, sclerotised rod and fourteen marginal hooklets of dissimilar shape and size. Whereas, in the genus Mizelleus haptor is trilobed connective piece, patch of peculiar shape and three types of marginal hooklets. Both are similar in having large dorsal hamuli and divided ventral bar but due to the presence of other different haptoral sclerites, there is slight difference in the adhesive mechanism.

Cerfontaine (1896 ,98), pointed out that monogeneans have specific preference for the ite of attachment on the gills. Subsequently, many other workers have also observed this phenomenon of preference of monogeneans on a particular gill arch. Frankland (1955) While working On another monogenean *Dactylocotyle denticulate*, observed heir preference for the Ist gill arch of the host Gadus river. This was further supported by Llewellyn(1956), who found that *Diclidophora merlangi* is attachrd to the first gill arch of *Gadus merlangus* and 2nd and 3rd gill arches of *G.luscae. Diplozoon paradoxus* prefers 1st and 2nd gill arches.

The basic pattern of locomotion, followed by these parasites was leech like. Unlike *Urocleidus adspectus*, as described by Cone and Burt (1982), it is notice that the locomotion of *Malayanodicoides* was quite slow and they move on the same side of secondary gill lamellae of a primary gill lamella. In case of Bychowskyella it moves on secondary gill lamellae but movement was fast.

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ETHICAL PRACTICE IN ANIMAL EXPERIMENTS, EXECUTION AND OBSTACLES: A REVIEW

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ABSTRACT

Increasing practice on animals in the research projects has paying attention towards the care, welfare and ethics associated to animal. The present review aims is highlighting the variety of aspects of animal care. Testing on animals is now been conducted in several research institutes. Animal testing have great practical consequentiality in finding a way for treating several diseases efficiently in humans as well, the reason behind this is very simple as human biology is very much similar to that of many other animals. Over 85% of animal research is regularly been conducted on mice, rats, fish, amphibians and reptiles. Rodents are the commonly used animal models, on which *in-vivo* studies are regularly been conducted because of their genetic, biological and behavioural homogeneous attributes with that of humans. Thus the present review objective is to provide an insight about appropriate route to be developed for animal experiment studies and their managment.

Key Words: Animal care, Ethical Practices, Animal experiments

INTRODUCTION

Animals are used at a large scale in many research institutions as test subjects for testing the efficacy of different treatment modalities for acquisition the knowledge on human disease [1]. Animals (mice and rats) have various physiological and genetic resemblances, thus ultimately furthering medical science [2]. Wide range of rudimental science questions, from physiology, immunology, pharmacology, toxicology, pabulum, deportment and learning have been solved by using rats as a laboratory species [3].

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With the end goal of Animals experiments, the Committee (CPCSEA) is shaped by means of the Act of the Indian Parliament, underneath the Prevention of Cruelty to Animals Act 1960 [4]. It is formed in 1964, restored in 1998, under chairpersonship of Maneka Gandhi. In the most current years, the CPCSEA had executed huge efforts for the better existence of the creatures in studies facilities crosswise over India [5]. This advisory group is made from individuals from established researchers, administrative professionals and creature activists [6]. The CPCSEA capacities with a wonderful system of volunteers who coordinate with the studies centres. [7]. With criterion for India: more than 665 labs are enlisted with the CPCSEA; Institutional Animal Ethics Committees (IAECs) are compulsory in every lab, which might be just enabled to suggest examine increase proposition that utilization rats, mice, guinea-pigs or rabbits; each undertaking that utilizations canines, ovines, bovines or non-human primates must be led if affirmed through the board of logical professionals constituted because of this; policies on lab creature care and practice had been defined and certified; a convention for the technology of immunobiologicals from horses has been planned and approved by the ultimate courtroom of India. The CPCSEA has been assessing on alternatives and operating out to offer alternatives in fundamental/administrative studies and guidance, almost about the commonplace field; the CPCSEA has restored and homed extra than three hundred canine, one hundred fifty horses, 2 hundred non-human primates and some dairy livestock, cat, winged creatures, mice and rabbits. The CPCSEA proactively prepares and directs logical and non-logical college on troubles of alternatives and lab creature welfare and it has battled lawful issues on lab creature care and make use of and have had choices that favoured picks and creature welfare [8-9].

It is important to develop a congruously standardized *in vivo* model for animal studies, while taking into concern animal welfare and the ‘3Rs’ principle. Rat models have several benefits availability of compulsory materials, simple techniques, high reproducibility, and practicality for experiments with immensely colossal sample sizes [10].

Experiments on animal are a difficult issue. In 1997 Dr Jay Vacanti & team grew an auditory perceiver on the back of a mouse [11]. Incipient medicines have been developed by conducting animal experiments to develop and for measure the safety aspect of the products [12]. These

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experiments are painful to animals also they reduces their quality of life [13]. If it is incorrect to cause animals to suffer, then experimenting on animals engenders intense moral uncertainties [14]. Animal experimenters should remember that during experimentation they should be very careful of this ethical issue and they should be as humane as possible [15]. They additionally allow that it's incorrect to utilize animals if alternative testing methods would create equivalent valid results [16].

In favour of animal experiments, suffering should be minimised and experiments should be utility based [17]. Animal experimentation is always unacceptable as it causes animals to suffer a lot. The animal experiments may engender benefits to humanity thus it is morally acceptable to harm few animals so as to provide moral justification [18].

"3 R's" principles i.e. Reduction, Refinement, Replacement are three basic principles to be followed for reducing the impact of research on animals [19]. Reduction refers to reducing the quantity of animals used in experiments by making better experimental techniques, data analysis and exchanging research information [20]. Refinement refers to using less invasive techniques, proper medical care etc. Replacement refers to alternative experimental methods i.e. cell cultures in place of whole animals, utilizing computer models etc [21].

Scientists express their view that excluding animal experiments would mean either a terminus to testing incipient drugs or utilizing human beings for all safety tests [22]. Experiments on animals are not only done to show the safety aspect of drugs and its efficacy in human beings, instead, they are acclimated to avail decide whether to test a particular drug on people so as to eliminate drugs which are ineffective or too perilous to utilize on human beings [23]. Drug passing the animal test, are tested on a human group before large scale clinical tribulations [24]. The significance of animal testing is demonstrated by a William D H Carey (pharmacologist) in a letter to the British Medical Journal, where they have used 4 possible incipient drugs to treat HIV [25].

Suitable studies based on animal experiments can benefit human beings for its applicability [26]. There are numerous scientists who oppose or are not convinced for conducting these tests typically claimed by proponents of animal experimentation [27]. Animal experimentation has

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been resulting in withholding of drugs from years [28]. Animal rights activists often update ethical rules on practitioners, who conventionally conduct cruelty during animal experiments, so as to part with any own moral standing [29]. The ethical status of the experimenters argues that the animal experimentation are morally right or wrong [30]. The general moral character of the experimenter is different.

The missing things of the experimenter in the ethical issues are ethical self-examination involving avoidance of animal suffering, which ultimately results in experimenters dehumanization along with ethical degradation [31]. Scientist like Gluck had offered their valuable opinions regarding animal experimentations [32].

The consumption of animals in research field should evolve out of a vigorous sense of ethical self-examination which involves a thorough estimation of one's own personal as well as scientific motives [33].

Animal experiments and rights

As it is wrong to break rights, it is important to understand that nature too has rights and if animal experiment breaks animal rights, then it is ethically wrong [34]. As animal rights should never be offended, animal experiments possible benefits to humanity are rude to the morality of the case [35]. As said by a philosopher, “there are some things that humanity will never be able to learn, so be it”. On the substratum of rights, deciding the morality of animal experimenting, people probably justify animal experiments on consequentiality grounds; by exhibiting that human benefits may involve animal suffering [36].

Justifying animal experiments

Experimenters favouring animal experiments leave an argument that the human benefit is larger than the harm done to animals [37]. As they visually examine the actions consequences under consideration. It’s not possible to prevent all experimental forms. Even advantages are fruitful to humanity as there are a few forms of suffering which might be probable infeasible to justify.

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Ethical arithmetic on animal experiments

By comparing ethical consequences of doing animal experimentation or no longer the demonstration of the consequentiality justification of animal experimentation can be carried out. The usage of such procedure cannot be done mathematically manner to avail humans determine moral questions in practice, but it does show the troubles very transparently. If appearing is very dangerous, then it's far ethically incorrect to carry out that experiment [38]. thus earlier than engaging in an animal test and for sake of animals, 3 matters should be stored under consideration, the ethical value of a person, secondly the wide variety of people who would have benefited and the last one the fee of the gain that every human being won't get [39]. however it is not so simple as it's without a doubt infeasible to assign a moral cost to a being, a cost to the harm executed to every individual. The damage with a view to be accomplished with the aid of the experiment, however the advantage is unknown the harm finished by using the experiment is due to an movement, whilst the harm as a consequence of no longer doing it's far because of an exclusion.

Certain versus potential harm

Comparison is made, by weighing two conceptually different things i.e. the animal harm versus human's loss by ceasing animal experiment [40]. The two things are different in the respect that animal harm during experiment is certain to occur if the testing is carried out. Instead of the consequence of animal experiments is unknown because it's not known how prosper the experiment would be or what profit it might generate. So it's a tedious job to draw a conclusion whether animal experiments are ethically acceptable [41].

Acts and omissions

The ethical difference present between omissions and acts does not dealt by using the equation. Ethicists cerebrate it morally worse or have opinion that we've got a moral responsibility concerning things we execute as opposed to matters we fail to do. Within the animal test context, the experimenter may damage the animals worried in experimentation but by no longer doing so the victim could be humans as they may not gain from a treatment for his or her disorder because of unavailability of the possible remedy [42].

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Consequently it may be verbally expressed that it's miles morally not good for the researcher to harm the animals through experimenting, than it is to (doubtlessly) harm a few humans via not doing an experiment that might discover a treatment for their ailment. And so if we pick out to maintain with the arithmetic that we began within the above phase, we require to position a supplement, and different, aspect on every aspect of the equation to address the specific ethical values of acts and omissions.

More approaches on animal experiments

Few examples confirmed that researchers have been organized for experimentation in a way that need to no longer have been sanctioned on animals [43]. And a small number of researchers have suggested that the equal benefits may be acquired through experimenting on people in place of animals. Indeed, given that uncertainty remains because scientists should expand the packages from animal models to human beings and suppose there are properly scientific motives for preferring human subjects. If human topics had been now not involved and capable of supply loose and apprised consent to the experiment then this might now not be morally objectionable.

The main changes proposed for animal experimentations are, to compulsorily carry out ethical reviews and require sanction for animal experiments, to include categorical invertebrate species, improvements to set minimum loss along with care, to require that only second or older generations animals of be utilized, to avoid using animals from the wild and exhausting wild populations, to state that alternatives to testing on animals must be utilized, to require member states to make the breeding, and utilization of the procedures so as to eliminate or reduce to a minimum any possible pain, suffering, distress or lasting harm caused to animals [44, 45].

Additionally exclude on utilizing great apes in scientific procedures, other than in exceptional situation, but there is no proposal to phase out the expenditure of other non-human primates in the immediate future.

CONCLUSION

For testing cosmetics or harsh products on animals there is no justification today. In situation, if it is absolutely indispensable for potentially preserving a human’s life then only animal experiment should be used. Alternative methods of animal experiments should be explored and

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Suffering should be minimized. Number of animals should be minimised in research. The amalgamated states should at least follow the standards of the European cumulation. Researcher should be vocal that we prioritize both the best research for preserving and rejuvenating humans and value for the animal life. This is what our tradition instructs us.

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AUTHORS CONTRIBUTIONS

The entire authors have contributed equally.

CONFLICT OF INTERESTS

There is no conflict of interest regarding publication of this review article.

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COVID-19 outbreak: An overview

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Abstract

In present scenario, the Corona virus has become a leading life-threatening disease. The virus has severely impacted the economy of developing and developed countries to a large extent. In December 2019, the disease outbreak due to novel corona virus SARS-CoV-2 had emerged in the Wuhan city China, has been spreading worldwide rapidly and World Health Organization (WHO) announced COVID-19 as an epidemic. Although, some countries have developed vaccines against the virus, still the second and third wave of disease has emerged. The objective of this review article is to have a preliminary outlook about the disease, the way of transmission, and diagnosis in the initial stages of COVID-19.

Keywords: SARS-Co-Virus-2, Corona virus, COVID-19, outbreak.

Introduction

The world is suffering from novel virus that posed serious threat to global health. In December 2019, Wuhan, Hubei province, China, become the hub of a pandemic of severe pneumonia cases were reported. First patients were admitted on 12 December, 2019, with symptoms of pneumonia, 27 cases of viral pneumonia with critical patients, were formally announced on 31, December, 2019 [1, 2]. Gradually, the number of patients began to increase with similar symptoms. Health professionals were unable to recognize the causative agent; therefore, these initial cases were classified as "pneumonia of unknown etiology". Centre for Disease Control and Prevention (CDC) centre of China organized a serious outbreak investigation programme. Thereafter, etiology of this severe illness is now recognized as a novel virus, belonging to the family of coronavirus (CoV). The virus was identified and named 2019 novel coronavirus (2019-nCoV). On 11th February, 2020, Commission of specialists of the International Committee on Taxonomy of Viruses (ICTV) coined it the SARS-Co-V-2 virus because it is genetic composition was similar to the virus that caused the SARS outbreak (SARS-Co-Vs). Later on, WHO has changed the name of the virus to Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) [3]. SARS-Co-V-2 is a novel virus accountable for an eruption of respiratory disease identified as COVID-19, which has now reached most of the

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countries. In the previous two decades, several pandemics like SARS-Co-V aggravated in China, involving more than 25 nations with 8096 confirmed cases and 800 deaths, in 2002-2003 [4, 5]. In 2009, cases of H1N1 influenza have been reported. In 2012, an outbreak of middle east respiratory syndrome coronavirus (MERS-Co-V), occurred in Saudi Arabia, claiming around 2,500 positive cases and 800 deaths [6].

This novel virus is highly infectious and contagious. Till March 10, 2020, it was spread in 109 countries; there have been 113,702 confirmed cases worldwide and 4,012 deaths have been recorded. Approximately, 71% (80,924) of all coronavirus confirmed cases and 78% (3140) of all death cases related to COVID-19 are from China. After seeing this worst situation, WHO declared China as a "very high risk" province for COVID-19 [7]. On March 11, 2020, WHO officially declared COVID-19 outbreak as a pandemic. Till August 09, 2020, there have been 19,462,112 confirmed cases globally, and 722,285 deaths have been registered [8].

Etiology

Coronaviridae is the family of Coronaviruses (CoV) is Classified into four genera of CoVs, namely; alpha, beta, delta and gamma Coronaviruses. The CoVs genome are RNA viruses consisting of positive-sense, non-segmented, single-stranded RNA with size range between ~27-32 kb, covered by an enveloped structure [9, 10]. The virus is either spherical or pleomorphic as evident in electron microscope. It is characterized by external trimeric spike of glycoprotein on its surface that displays as crown-shape with 80-120 nm in size [11, 12, and 13]. The genomic RNA contains a 5` cap and 3` poly (A) tail and multiple open reading frames (ORFs), allowing it to act as an mRNA for translation of the replicase polyproteins.

Among all the RNA viruses, the RNA genome of CoV is one of the largest [14]. With its high mutation rate, Coronaviruses have become the leading cause of respiratory disease outbreaks. SARS-CoV-2 is the 7th Coronavirus identified to infected humans; SARS-CoV, MERS-CoV and SARS-CoV-2 can cause critical disease, whereas NL63, 229E and OC43 are linked with mild indication [15]. Biochemical and structural studies revealed that the (RBD) Receptor Binding Domain portion of the SARS-CoV-2 spike proteins was so effective at binding to the ACE2 human receptor [16, 17]. Genomic based studies have shown that SARS-Co-V-2 has probably originated from bats and rodents and are the two notable genomic sources of alpha and betaCoVs [18, 19].

Coronavirus Replicating in human body

The cells in the lining of the nose are rich in the cell-surface receptor angiotensin-converting enzyme-2 (ACE-2). ACE-2 receptors are present in ciliated epithelial cells in the lower and upper airway. The

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virus requires this receptor to enter a cell and releases its RNA. The virus RNA uses the host cell to generate new virus RNA and assemble new virions [20].

Four proteins, RdRp, PLpro, and 3CLpro spike are essential for the replication of virus. Therefore, therapeutics targeting one of these proteins is at present being tested as a probable treatment for Covid-19. The spike protein of SARS-Co-V-2 is drastically diverse from SARS-Co-V spike, particularly in two sites when binding to ACE2. Therefore, formerly developed antibodies and remedial peptides for SARS-Co-V spike protein cannot be used for SARS-Co-V-2.

Transmission of Coronavirus

The first confirmed case of the COVID-19 was related to direct contact to the Wuhan Seafood Market, the animal-to-human broadcast was assumed as the main method. Consequently, it was accomplished that virus could also be transmitted from human-to-human. The transmission is supposed to occur through respiratory drop from sneezing and coughing. Currently, it remains indistinguishable whether a person can be infected by COVID-19 by touching an exposed object or surface and then touching their nose, mouth, or maybe eyes [21]. According to data analysis related to the SARS-Co-V-2 spreads in China seems to point out that close contact between individuals is necessary.

Strange history of this novel disease

The clinical history in some patients occurs with particular characteristics. It foresees that the patient has fever, which is not extremely responsive to antipyretics, and a state of depression. A dry cough is regularly associated. After 5-7 days, patients with impaired lung function start to experience shortness of breath and increased respiratory rate. In more delicate patients, however, bronchial crises may appear at the beginning of symptoms. In younger patients and in those who do not have critical respiratory impairments or other comorbidities, dyspnea may appear later. Infected patients experience decline lung injury, there is a decline level of oxygen as well. This seems to be the critical phase of the disease, from this point onwards; there may be rapid impairment of respiratory functions. The situation is truly incredible because for patients who are slightly hypoxic and paucisymptomatic, the first therapeutic approach is oxygen therapy and next is mechanical ventilation. Although this approach is effective, still deterioration of respiratory failure may occur in some patients.

Symptoms and severity spectrum of COVID-19

Patient diagnosed with COVID-19 may be infected from the coronavirus fourteen days before as its incubation period in host is 14 days. Common symptoms of COVID-19 are dry cough, fever,

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tiredness and shortness of breathing. Doctors are also noticed that in some cases, sudden loss of smell and taste are early indications of novel coronavirus infection [22].

Clinical progression-Diagnosis

Before SARS-Co-Virus cases, it was considered that human Co-Virus leads to normal cold, or superior and inferior respiratory infection. In the past years, similar to SARS-Co-V, MERS-Co-V that caused epidemics, first symptoms of illness were commonly distinct as cough, cold, fever, shortness of breath [23]. Early diagnosis of 2019 n-CoV infection is required to manage the disease. Till date, two methods are available for the early and accurate detection of virus.

1. Real Time Reverse Transcription-Polymerase Chain Reaction (RTi-RT-PCR)

Polymerase Chain Reaction has been established as 'gold standard' in the detection of disease-causing pathogens. Various platforms of PCR have been developed for specific and sensitive detection of target pathogen. In organisms where genetic material is RNA, reverse transcription real time PCR is used to detect RNA and quantify the presence of causative pathogen/organism. Real-Time RT-PCR uses different probe chemistries for the quantification of target nucleic acid. The technique offers great sensitivity for diagnosis. By virtue of these RTi-RT-PCR is used for the early (~ 3-4 hours) and sensitive detection of the causative agent of COVID-19, SARS-CoV-2 [25].

2. Lateral flow / Colloidal Gold Immunochromatography

Immunoassays based detection system has been developed for quick detection of SARS-CoV-2. Lateral flow immunoassays are in application for the rapid screening. These assays provide even bedside diagnostics in hospital settings. In particular, SARS-CoV-2 virus or developed antibodies (IgM & IgG) against COVID-19 are detected using this assay. Although, the method is rapid, still there are some disadvantages associated with this. Poor specificity and sensitivity are major challenges of this method [26].

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Drug repurposing for antimicrobial activity on selected bacterial species

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Abstract

Increasing cases of multidrug-resistant pathogens have evolved into a global health crisis. *Salmonella sp.*, *Proteus sp.* and some strains of *Bacillus* especially isolated from soil are pathogenic and harmful to human health. Antibiotics are usually used in the treatment of selected bacterial species are fluoroquinolones (Ciprofloxacin, Azithromycin and Cephalosporins) for *Salmonella species*, Clindamycin, Vancomycin, Cephalosporins and Penicillins are effective against *Bacillus* infections and Nitrofurantoin, Tetracycline, Polymyxins, Ceftriaxone, Quinolone, Gentamicin (plus Ampicillin) are used in the management of *Proteus species*. These bacteria are also associated with antibiotic resistance, and infections caused by pathogens result in high mortality and morbidity. Thus there is a need of drug repurposing and our study gives the choice of drug for effective treatment by repurposing already known and tested drugs against the bacterial infections. In present study four antibiotics (Azithromycin, Ciprofloxacin, Doxycycline, Ofloxacin), one anti-malarial (Quinine) and one anthelmintic (Albendzole) agents were screened for repurposing of antibacterial activity on three different species of microorganisms i.e. *Salmonella sp., Proteus sp.* and *Bacillus sp.* The multiple drug resistance of *Salmonella sp., Proteus sp.* and *Bacillus sp.* against tested drug concentrations was evaluated by using disk diffusion method. Antimicrobial activity was confirmed by the presence of zone of inhibition. The test results indicated that Ciprofloxacin and Doxycycline worked on all the three selected species. It was also found that Quinine and Albendzole were effective against *Salmonella sp.*, and *Proteus sp.* After that we selected different ratio of these two drugs and found that Ciprofloxacin with Doxycycline at 1:1 proportion gave best zone of inhibition for all the three bacterial species that was 30 mm, 35 mm and 37 mm, respectively than the individual activity of each drug. Thus, our study emphasizes on the easy repurposing of available antimicrobial agents which will be cost effective and less time consuming.

Keywords: Drug repurposing, Antimicrobial activity, Antibiotics.

Introduction

Drug repurposing is a secure pathway to speed up successful development of the novel drug. This process uses either FDA (Food and Drug Administration) approved drugs or drugs that are failed in clinical trials. These drugs have its detailed information on formulation, potential toxicity and

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pharmacology. Due to scientific advancements in the biological sciences, informatics and genomics, it is possible to identify secondary response of existing drugs in drug repurposing. Drug repurposing have significant role within the finding of various possible antibiotics. Microbial infection like bacteria emerged as a growing public health threat by multidrug resistance for current drugs at worldwide level. This increases the urgent need of improvement of new antibiotics that may successfully fight multidrug-resistant bacterial infections (MDRBIs) (1).

Different Institutions identified that antimicrobial resistance is a worldwide health risk that has compounded through the reduction in the discovery and improvement of new antimicrobial agents (2,3). Consequently, the development of recent antimicrobial therapeutic strategies requires immediate attention to keep away from the ten million deaths predicted to occur by 2050 because of multidrug-resistant (MDR) bacteria. Despite the increasing interest in the improvement of repurposing drugs, only few repurposing drugs are below scientific improvement towards Gram-negative pathogens (4).

Drug repositioning also termed in means by their motif such as restoring, re-profiling, therapeutic switching, re-purposing and its side activities. As the existing drug have gone through testing procedures, this shortcut method of re-utilizing can reduce the cost, time, risk and various known parameters in concern. Archived drug that failed to step into market but have safety and risk clearance have also shown potential to repurpose (5,6).

Material and Method

Isolation and identification: Bacterial species isolated from soil sample were biochemically tested and identified recommended in the Bergey's Manual of Determinative Bacteriology (7). Then these strains were maintained by incubation at specific time and temperature on growth media for *in-vitro* investigation in our institutional laboratory. Further commercially available antibiotic disk diffusion test was performed to detect sensitiveness, intermediate inhibitory zone and resistance. This was detected using following antibiotics - Tetracyclin, Gentamycin, Ampicillin, Co-trimoxazole, Cefuroxime, Ofloxacin, Erythromycin, Oxaclin, Cefaclor via disc diffusion method. All the drug resistant strains were then grown at 37^0C (98.6^0F) in nutrient broth and subcultures were made freshly to run subsequent experiments. Proper sterile conditions were maintained and good laboratory procedures were followed while dealing with these multidrug microbial strains.

In the present study four antibiotics (Azithromycin, Ciprofloxacin, Doxycycline, Ofloxacin), one anti-malarial (Quinine) and one anti-helmintic (Albendzole) agents were screened for repurposing of antibacterial activity on three different species of microorganisms i.e. *Salmonella sp., Proteus sp.* and *Bacillus sp.* (8).

Antimicrobial sensitivity test: Incubation procedure - Under aseptic condition using sterile swab dip into broth medium of test organisms (target bacterial strains) then gentle pressing of swab end against

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the inside wall of tube to decant excess soaked liquid, bacterial lawn on Muller-Hinton agar plate was prepared by swabbing in uniform manner in all direction.

Using agar wells diffusion technique wells were punched with diameter of 2 mm in the previously inoculated test bacterial culture on Muller Minton agar plate. Minimum Inhibition Concentration (MIC) was determined by micro-dilution of sample (test) drug in sequential decreasing order of product concentration (100, 50, 25 and 12.5ug/ml). Observations were made after incubation at 37^0C for 20hrs.

Visible inhibitory growth of lowest concentration of test drugs (MIC) was determined by measuring the diameter using ruler template for zone of inhibition (8).

Combination of Drug for repurposing screen assay: Multiple drug agents showed improved effect when used together against antibiotic resistant bacteria (9). Well diffusion method was used for this screen assay (8). Mechanism underlying in this event maybe based on the chemical interaction, inhibitory action with dual force or other unknown factors.

Micro-dilution was done in two ratios (1:1 & 1:2) with reciprocal dilution by using minimum inhibition concentration of test drugs previously studied and measured value estimated.

Combined effect on varied antimicrobial induction was done on the test microorganisms with freshly broth culture incubated at 37^0C for 24 hrs, plated on Muller Hinton Agar and the result was measured by the zone of inhibition dimension. Comparative study of single drug and two drugs effect was done to detect the likelihood of synergism.

Result

The antimicrobial activity was assessed following standardized method. The manual estimation of zone of inhibition by measuring the diameter circling the clear zone is easy and feasible. Comparative antimicrobial activity of antibacterial analysis has done for Azithromycin, Ciprofloxacin, Ofloxacin, Doxycycline, Quinine (anti-malarial drug) and Albendazole (anthelminthic drug) against selected pathogens. Table 1 shows standardization of above drug concentration against the different species of test bacteria.

Table 1: Standard concentration of drugs used in the present study against the test pathogens:

Test Bacterial Strain	**Azithromycin (µg / ml)**	**Ciprofloxacin (µg / ml)**	**Doxycycline (µg / ml)**	**Ofloxacin (µg / m l)**	**Quinine (µg / ml)**	**Albendzole (µg / ml)**
Salmonella sp.	25	12.5	25	12.5	25	200
Proteus sp.	-	12.5	100	12.5	12.5	-
Bacillus sp.	-	12.5	100	12.5	-	-

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Combined effect of two different drugs was tested against bacterial strain that showed resistance against other choice of antibiotics. Individual minimum inhibitory concentration towards test drug was compared with collective drug result in which we have found that ciprofloxacin with doxycycline at 1:1 proportion gave best inhibition zone for all the three selected bacterial species that was 30 mm, 35 mm and 37 mm, respectively. Thus it was concluded that ciprofloxacin in combination with doxycycline increased antibacterial activity against tested Salmonella *sp., Proteus sp.* and *Bacillus sp.* (Table 2).

Table 2: Minimum inhibitory concentration of different antibiotics (individual & combined) used in the present study:

⬇ ➡	**Name of Bacterial species**	***Salmonella sp.***	***Proteus sp.***	***Bacillus sp.***
S.No	**Name of Drug**	**Zone of Inhibition (in mm)**		
1.	**Azithromycin**	**15**	**-**	**-**
2.	**Ciprofloxacin**	**32**	**32**	**34**
3.	**Doxycycline**	**15**	**18**	**17**
4.	**Ofloxacin**	**21**	**18**	**-**
5.	**Quinine**	**16**	**14**	**-**
6.	**Albendzole**	**21**	**-**	**-**
7.	**Ciprofloxacin + Doxycycline (1:1)**	**30**	**35**	**37**
8.	**Ciprofloxacin + Doxycycline (1:2)**	**26**	**34**	**37**
9.	**Doxycycline + Ciprofloxacin (1:2)**	**30**	**34**	**36**

Discussion

Antibiotics are the important agents in combating microorganism infections. Drugs play a major role within the interference and treatment of human diseases. The microorganisms like bacteria have the genetic ability to spread and gain resistance to synthetic drugs that are used as therapeutic agents.

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Despite ongoing efforts to identify new drugs or alternatives to antibiotics, no new classes of antibiotic or their alternatives have been clinically approved in the last three decades. A combination of antibiotics and non-antibiotic compounds that could inhibit bacterial resistance determinants or enhance antibiotic activity offers a sustainable and effective strategy to meet multidrug-resistant bacteria (10-12). Drug-drug interactions can be divided into three types: synergy; no interaction; and antagonism (13).

In the present study, efforts have been taken to find out any kind of positive interaction among the common antibiotics which may cover the pathway for repurposing of these drugs against the drug resistant pathogens which cannot be treated by following the conventional treatment strategy. Ciprofloxacin and Ofloxacin are known to cause damage in bacterial cell through DNA manipulating pathways. Whereas anti-bacterial drug Azithromycin and Doxycycline also known remedy for malarial infections. The definite mechanism of quinine is still not discovered yet. Azithromycin and anti-parasitic agent Albendazole gave better results for Salmonella species. All the three selected bacterial strains of *Salmonella species*, *Proteus sp.* and *Bacillus sp.* showed sensitivity against Doxycyline and Ciprofloxacin. These antibacterial medicines are counted among the world bestselling drugs. But the generation of multidrug resistance into existence is one of the top most threats to human kind. The pace of adapting for defense mechanism by the microbial community is spontaneous and unpredictable. The outcome obtained in the present study indicated that how the drugs included in the study can show activity on other microbes also for which they are not normally prescribed. Our findings were found according to various other similar type of studies (14-17). The synergistic action of two drugs Ciprofloxacin and Doxycycline was reported by the increased zone of inhibition as compared to their individual action against the tested pathogens. Thus the results indicated positive aspect of using these drugs in combination in different ratios and also paved a way to repurposing treatment process using these drugs.

Conclusion

The use of antibiotics for treating any kind of infection or disease is very common these days. Their use often goes unmonitored or uncontrolled. However, current antibiotics became less effective as a result of the emergence of drug-resistant microorganisms. It is imperative to research newer drugs that are active against drug resistant microorganisms. The drug repurposing results in quicker, cost effective and easy approach to deal with the problem by giving new useful roles to the already known drugs against a different microbial community. The present study was a successful trial for the same and the results are found to be very optimistic in drug repurposing field.

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Author's contributions

The entire authors have contributed equally.

Conflict of interests

There is no conflict of interest regarding publication of this article.

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Nano-fertilizers and their role in plant nutrient management

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Abstract:

The conventional fertilization system is now not suitable in the present agriculture system due to limited resources and low use efficiency, and costly fertilizers and other environmental associated factors. The development of improvised fertilizers using nanotechnology is the most potential approach, which includes development and improvisation of nanofertilizers. Nanofertilizers utilizing in crop fertilization process increases crop production substantially. Nanofertilizers acquire some characteristic features which contribute to develop of precision base agricultural. Application of nanofertilizers cause increased efficacy of the micro and macro elements made available to plants growth and development. The use of nanofertilizers offers a several benefits over conventional fertilizer in present agriculture system, importantly nanofertilizers exhibit slow and regulated release of nutrients into the soil leading to nutrients effective availability. Nanofertilizers help to optimize nutrient management and facilitate higher nutrient use efficiency at various developmental stages in plants, causing more yields in contrast of traditional chemical fertilizers. The advances of nanobiotechnology offer several opportunities in agriculture system for optimum utilization of resources, nutrient management and crop production. The value addition in nutrient content and quality in food crops is another area that is least explored, the potential role of nanofertilizers in augmenting food nutrient value through nutrient fortification in several crops are suggested by various studies. In the recent years, various reports suggested the potential benefits of nanofertilizers in precise agriculture system that supports the increased nutrient uptake, soil nutrient enhancement, improved food nutrient quality, minimize nutrient loss, and also reduces the soil toxicity because of the heavy application of the traditional fertilizers in present agriculture system.

Key words*:* Nano-Fertilizers, nutrient use efficiency, crop production, nutrient biofortification, sustainable agriculture

INTRODUCTION

The present agriculture scenario needs most concern to enhance agriculture production to cater increasing food requirement to feed the growing global population. It is also very much important to develop different technologies and strategies in the present agriculture system to boost crop

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productivity and support agriculture sustainability. In the last decade, nanotechnology has emerged as promising technology in this sector to cater effective agricultural crops management and boost crop production. The crop nutrient management is one of the area, that needs to be extensively explored, and nanotechnology could provide plausible solution in this direction. Crop fertilization is very crucial practice in agriculture system required for more crop and better yield; therefore, it is important to look for suitable technologically evolved approaches in the agriculture system for the fertilization process for easy bioavailability of nutrient to the crops. This will increase the crop yield in an eco-friendly manner [1]. The present agriculture system is heavily using synthetic agrochemicals which include pesticides, herbicides and synthetic fertilizers. Such agrochemicals are used in agriculture for crop protection and to improve crop growth and production. As per the recent report of FAO, the global production of synthetic fertilizers was about 118.2 Mt in 2019 [2], and is expected to continuously rise in future also to cater for the growing need of food demand by 9.6 billion people globally by 2050, awhich is a big challenge to the scientists. [3]. However, the current agricultural practices in the agricultural system are not sufficient enough to use the fertilizers in their actual capacity, and also are not able to give sufficient thrust to enhance NUE, and uptake and bioavailability of nutrients to the plants, resulting in low crop production less utilization of resources [4]. There are studies on the nutrients used by plants revealed that conventional chemical fertilizers are hardly utilized to their maximum potential in exiting the agriculture system. Most of the important nutrients (including both macro and micro) available in the fertilizers are underutilized due to less NUE and wastage through diverse means including leaching, degradation, immobilization, hydrolysis etc. For example, some of the important nutrients like, Nitrogen, Phosphorous and Potassium are hardly used by the plant to as low as 30–35%, 18–20%, and 35–40%, respectively [5].

The prolonged use of chemical fertilizers in traditional agriculture system extensively to improve nutrient content in soil and also soil fertility has started showing adverse impact and reduced soil fertility along with water quality. Conventional nitrogen fertilizers due to their less availability to plants cause an adverse impact on the environment triggering eutrophication of water bodies, increasing soil acidity and loss of soil biodiversity [6] [7].

In recent years, the focus is shifted on nanotechnology in the agriculture sector to explore new technology development and innovative approaches to boost agricultural sustainability. Nanotechnology offers potential approaches towards agricultural sustainability through several ways including effective use of agrochemicals, increasing efficiency and minimizing the loss of resources. The nano-materials are proved to be an important carrier for agrochemicals and facilitate nutrients supply to plants through regulated manner and witnessed enhanced nutrient use efficiency that translated into more crop production [8, 9]. The development of nanofertilizers for contemporary agriculture system can provide a potential boost to agronomic sustainability through diverse

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approaches i.e, slow delivery of nutrients in a controlled way, and more effective nutrients bioavailability, enhanced nutrient uptake and NUE, further, it is corroborated with resource conservation, reduced nutrient leaching and nutrients loss in environment etc. nutrients loaded nanofertilizers, offer sustained and slow nutrients delivery so that it can be made available to plants for better utilization under normal conditions. Nanotechnology offers huge potential to give a boost to crop production and promote food security in many ways including crop fertilization, which is the most promising practices to enhance crop productivity. The huge and indiscriminate application conventional Nitrogen fertilizers for agriculture purpose put additional burden on the environment adversely by contributing in the accumulation of atmospheric N_2O, which is one among the different greenhouse gases [10]. The nutrient use efficiency in the present agriculture system is still very low. Despite several efforts, more than 50% nitrogen used in agriculture for crop fertilization lost and contribute to the environmental problem [11, 10].

According to EPA 2017, agricultural practices also contribute to emit around 10% greenhouse gases of all greenhouse gas emission; therefore, taking into the fact count there should be much concern in this regard to reduce the environmental impact [12].

Nonmaterial based fertilizers are more effective as compared to conventional synthetic fertilizers, due to their high surface area to volume ratio, letting them increase nutrient use efficiency and low quantity of application, leading to reduce loss of fertilizers released into the environment [13]. Nanofertilizers loaded nutrients can be uptake by root through different routes and means, which includes, ionic channels, aqua-porins, through transport proteins, and by endocytosis etc. [14].

Agriculture is the principal source of food to cater to the nutritional need of the population by the cultivation of different crops. The nutritional value of food is very important to determine the health status of the people. Therefore, there is a strong relationship between the food and its nutrition content to the people. Moreover, the low nutritional value of food especially with less or no micronutrients is an important issue and cause adverse health issues especially to the people who have no alternative source for nutrient supplement [15]. Therefore, the nanofertilizers could be an alternative approach to add nutritional value to the important staple crops to address the challenges on malnutrition [16].

This article focus on the nanofertilizers and their potential role in the present agriculture system, further, the use of different nanoformulations and underlined mechanism to increase crop productivity, soil fertility, NUE, and enhanced food nutrient values or increased nutritive value etc. moreover, it will lower the synthetics fertilizers requirement and promote environmental sustainability and help toward agricultural sustainability with more yield and better nutrient food quality to deal with malnutrition.

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NANOFERTILIZERS

Nanofertilizers are defined as the materials that are composed of materials exist in the nanoscale level. They are mostly nanoparticles. They carry the important plant nutrients and deliver them to the crop plant in a sustainable and controlled manner [8].

The four important groups of nanofertilizers are classified as (i) Macronutrient nanofertilizers (ii) Micronutrient nanofertilizers (iii) Nanomaterial enhanced fertilizers, (iv) Plant growth-stimulating nanomaterials [13, 17]. Nanofertilizers (NFs) usually take 40-50 days to deliver nutrients, which can synchronize to the pace with plant nutrient uptake ability. While conventional chemical fertilizers quickly made most of the nutrients available to the soil (within 4-10 days) causing loss of nutrients through many ways, including leaching and loss in the environment [18]. Nanomaterials loaded with nutrients increase crop production by increasing nutrients availability in soil and nutrients uptake. Moreover, nanofertilizers also facilitates to boost crop productivity by increasing germination of seed and other plant physiological and metabolic attributes including photosynthesis and protective responses against both biotic as well as abiotic stresses[19].

Further, nano fertilizers based on their development and formulations are classified into three types i.e., Nanoscale fertilizers, nanoscale coating fertilizers and nano-additive fertilizers. [20].

Nanofertilizers can slowly dispense nutrients in optimum quantity to target organelles in plants to facilitate nutrients to plants out of it, in the ecofriendly manner [21]. Nanoscale fertilizers are composed with nutrient nanoparticles, whereas in nanoscale additives fertilizers nutrient nanoparticles are supplemented to traditional fertilizers to increase nutrient uptake in plants. Further fertilizers coated with NPs were classified as nanoscale coating fertilizers. Even the encapsulation of nutrients is also one of the common approaches to produce nano fertilizers [22]. The targeted nutrients are packed (encapsulated) into nanoporous material rendering nutrient to diffuse in a regulated manner, or the nutrients coated with fine polymer film composed of nanomaterial offers another strategy for effective nutrient delivery to plants in a regulated manner. Additionally, the development of improvised nano-formulations further helps prolonged use and regulated delivery of nutrients for effective and balanced uptake by the plant to sustain productivity and also minimize nutrient loss [23, 24].

The macronutrient nanofertilizers are mainly composed of macronutrients (i.e., N, P, K, Mg, and Ca) whereas micronutrient nanofertilizers constitutes micronutrients ie. iron, copper, zinc manganese and molybdenum etc. Micronutrients are required in very trace amount and yet very essential for crop growth in association with macronutrients. The modern agriculture system needs to develop and produce eco-friendly macronutrients formulations, especially nitrogen and phosphate nanofertilizers for sustainable crop production by replacing them with traditional synthetic N fertilizers. The role of nanofertilizers become crucial for regulated nutrients delivery to plants even under adverse and stress full conditions [21].

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It has been observed in several research findings that, the use of micronutrient-containing NPs indicated that they could be a better option to enhance crop yield probably by the better supply of nutrients to the plants. Nanofertilizers may be formulated using different types of nanoparticles alone or in combination, they may consist of zinc oxide nanoparticles (ZnONPs), silica, iron and titanium dioxide, ZnS/ZnCdSe core–shell quantum dots (QDs), InP/ZnS core–shell QDs, Mn/ZnSe QDs, gold nanorods, Al_2O_3, TiO_2, CeO_2, and FeO [25]. Nanofertilizers are mostly studied on metallic nutrients like Cu, Mn, Zn, and Fe. Also, Al, Ce,La, and Ti and oxides of metals ZnO, TiO_2, Fe_3O_4, CeO_2, Au, Ag, Cu, and Fe nanoparticles., were also used as nanoparticles in plants for their positive effect on soil fertility and nutrient enhancement/ fortification in plant [26].

The nutrients that immobilized or encapsulated in nanocarrier are activated and released by three important factors i.e., biological factors, physical factors and chemical factors, which cause the the biodegradation of coating, solubilization of material or pH variation, soils types causes the effective release of nutrient [27].

NUTRIENT USE EFFICIENCY

Several observations and reports consolidate the idea of enhanced nutrient use efficiency in plants. It important if plants are able to utilize the nutrients effectively, the yield is going to be increased even under unfavorable conditions. The nanofertilizer formulations consisted of vital nutrients when applied to crops using any of the several ways to facilitated nutrients resulted into effective nutrient management and improved yield. The high Nutrient uses efficiency (NUE) could be attributed to better nutrient transport and effective delivery through plasmodesmata (nanosized channels) in plants usually 50-60 nm, the carbon nanotubes and silica nanoparticles are attributed to effectively transport and delivering different cargos to the target site in plants. Nanofertilizers have shown high nutrient use efficiency by plant resulting into increased nutrient uptake as plant cell wall have small pore size of up to 20 nm range [28]. Moreover, The extremely porous plant roots at the nanoscale primarily involved to uptake the nutrient from the soil, the process and efficacy of nutritional uptake is depended upon different factors for example plant morphology, growth stage, particle size, availability and exposure time etc. therefore, uptake of nanofertilizers using plant roots system can be enhanced using root exudates, molecular transporter via ionic channels and even creation of new pores or ion channels or even by exploitation of endocytosis process [26].

The development of nanonutrient formulations and their role application in crop nutrient management system and effect on crop yield is a potential approach to enhance crop productivity and reducing quantity of high doses of chemical fertilizers without affecting the crop yield, the increased NUE and improvised uptake of nanofertilizers have certainly boost agricultural productivity [29]. Nanofertilizers have been projected as a most promising and effective technological advancement in contemporary agriculture to cater for the growing need of food demand through controlled crop

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nutrient fertilization and improved nutrient use efficiency. The use of nanofertilizers can raise crop production to 30% as compared to traditional fertilizers [30].

There are various studies revealed that phosphorous and iron are important nutrients belonging to macro and micronutrient category respectively. They are essential for optimum growth and development, therefore, the low availability of such nutrients result in poor plant growth and low productivity. Sega et al 2020 conducted a study on cucumber and maize and revealed improved yield and growth when fertilization was achieved through the application of $FePO_4$ nanoparticles, it provides better NUE and bio availability of both nutrients to crop as compared to their conventional non-nano fertilizer[31].

The application of nanofertilizers in modern agriculture system provides an important strategy for nutritional fertilization of crops with minimum loss. Recently in the study by Miranda-Villagomez et al. 2019, indicated the nano-particles carrying KH_2HPO4 exhibited the increased physiological efficiency of phosphorous in root and shoot leading to higher biomass accumulation and increased instant water use efficiency in rice plants.[32]

Nano-fertilizers in several ways facilitate the effective nutrient uptake by plants to increase nutrient use efficiencies by using the unique properties of nanoparticles. The different nano-fertilizers formulations can be developed either by using a single or a combination of different nutrients at the nanoscale. The nanoparticle can be synthesized by adopting various strategies by applying any of the physical (top-down) and chemical (bottom-up) methods, and the desired nutrients can be used as it is for cationic nutrients (NH_4^+, K^+, Ca^{2+}, Mg^{2+}) and after surface modification for anionic nutrients (NO_3^-, PO_4^{2-}, SO_4^{2-}). Several studies revealed that nano-fertilizers can extend nutrients delivery to the plants even beyond 30 days of their application to the plants, which will provide balanced crop nutrition and also foster improved NUE. Which not only enhance crop growth but also crop yield [33].

It was reported by Yogendra et al 2020, the application of nano nitrogen fertilizers in crops raised the nutrient used efficiency to a significant level in crops and also helped to minimize the resources and save 50% of urea use, therefore the application of Nitrogen Nanofertilizers in agriculture sector for crop fertilization could be an important and novel approach to increase nutrient used efficiency and reducing the dependence on synthetic fertilizers especially nitrogen with lowering the impact on the environment[34].

ENHANCING FOOD NUTRIENT VALUE

In recent years, the scientific community has also started focusing in the direction of increasing the food nutrient value in crops, because the nutrients deficiency in food crops is affecting human health.

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Despite having other methods to augment the food nutritional value like dietary component diversification, application of drugs and other commercial fortification methods, such, methods are not enough and costly as well, therefore, nanofertilizers could offer potential benefits to the fortification of food nutrients in important crops. Moreover, different investigations reports indicating that plant nutrient contents were improved when appropriate nano fertilizers were applied to crops, as nutrients from fertilizers can be easily absorbed by the plant to fortifying the nutrient value of food. Appropriate soil fertilization in agriculture sectors is well accepted practice to fulfil the optimum nutrient need by plants for optimum growth and yield. The nutrients required by the plants are provided by use of suitable fertilizers; therefore, nanofertilizers with plant nutrients could be a potential tool to increase food nutrients value [16]. Agriculture is the principal mean of cater human nourishment, therefore food quality and its nutritional value hold importance to a humans health. So if staple food crops are having a poor or low nutritional value in food that could be an important concern regarding poor health relates issues especially related to malnutrition and also imparts adverse effect on socio-economic conditions [35, 36].

Fakharzadeh et al., 2020, recently studied that the application of nano chelated iron fertilizer increases productivity by 27%. Nano chelated iron fertilizer increased biological yield by 27% and along with a 13% increase in protein content. Moreover, the increased content of nitrogen, phosphorus, iron, potassium, and zinc were reported in white rice. It is clearly showing the capability to bio-fortify crops with vital micronutrients in rice [37]. In the other study, the nano chelated iron fertilizers applied on pistachio trees for nutritional fertilization, reported having enhanced quantity of iron content and calcium concentration along with substantial enhancement of soluble sugar content in pistachio trees [38]. Davarpanha, et al., 2016 reported enhanced nutritional quality and yield of pomegranate (*Punica granatum* cv. Ardestani.) by the application of boron and zinc nano-fertilizers [39]. Dapkekar, et al., 2018, reported having high zinc content and high protein content in wheat grain of durum wheat genotypes by applying the Zn-CNP nano-fertilizer. Moreover, it was suggested the utility of Zn-CNP as a novel nanofertilizer which also increase fertilizer use efficiency. Therefore, the approach of ferti-fortification using Zn-CNP nano-carrier could be and useful tool for other crops also. Further, such reports supported the enhanced yield and also nutritional fortification in various food crops by the application of nanofertilizers [40].

CONCLUSION

Nanotechnology is an emerging technology for precision agriculture, which offers the most potential solutions to enhance crop yield component and plant's nutritional value with minimum resource utilization. Nanotechnology offers technology to support agriculture management system although

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the technology is in the juvenile phase in agriculture. The application of nanofertilizers primarily conserve natural minerals and provide regulated and balanced nutrition to crops through enhanced nutrient uptake augmenting the nutrient use efficiency (NUE). Nanofertilizers also have a greater role in the prevention of nutrient loss, water contamination and negative effect on the environment. The nanofertilizers could be helpful for effective crop fertilization and better nutrient utilization in order to get more yields and high food nutrient value, which can helpful in addressing the issues of malnutrition worldwide. The identification, improvisation and development of suitable nanofertilizers formulations for crop nutrient enhancement come up as an innovative strategy concerning other available technologies. Further, it is also important to explore the extensive information on properties and other attributes the nonmaterial needed to get the maximum benefits. Although the nanotechnology is getting more attention to be applied in the agriculture sector to manage agriculture with more precision to address food security, at the same time, it is very crucial to monitor and evaluate the effects, advantages and adversities of nanomaterials on crops and impact on environmental safety, toxicity and other related issues.

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Herbal Medicine as Paradigm for Hepatoprotection: A Comprehensive Review

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ABSTRACT

India is sitting on a gold mine of well-recorded and well-practiced knowledge of traditional herbal medicine. Herbal medicine these days has become more popular partly because of scientific validation of some of their medicinal values. It is still an important pillar of about 75–90% globally, primarily in the developing countries, for essential medical care as a result of well social acceptability, superior compatibility with the human body. There are thousands of plant species having good potential of offering direct therapeutic effect individually or in combinations. Herbal medicines are considered as state-of-art laboratories proficient of biogenic synthesis of nanoparticles through different chemical classes. Many of these are proved to be precursors for development of other drugs. Furthermore, many western drugs have their origin through herbal extracts. Numerous herbal agents, effectively used for gastrointestinal, cardiovascular, nervous and metabolic disorders. A vast majority of plants has been examined for hepatoprotective potential, many of them with well-known proven capacity to combat hepatic dysfunction. Therefore, an attempt to harness the potency of plants to develop new hepatoprotective drugs has become the need of the era.

***Keywords*:** Herbal Medicine; Bioactive compounds; Hepatoprotection

Introduction

Plants assume a crucial part in sustaining health of anthropoid and contribute towards progress of human existence. They are significant segments of medications, beauty care products, colors, refreshments, dyes and so forth. As of late, center around herbal research has been expanded everywhere on the globe immensely. There are thousands of plant species having good potential of offering direct therapeutic effect individually or in combinations. Plants are considered as state-of-art chemical laboratories fit for biosynthesizing number of biomolecules of various synthetic classes. Many of these are proved to be precursors for development of other drugs [1]. Furthermore, many western drugs have their origin through plant extracts. Numerous herbal drugs, which are effectively castoff for gastrointestinal, cardiovascular, nervous and metabolic disorders. Ethno-botanical and ethno-pharmacological studies on such plants continue to attract investigators throughout the world [2].

Medicinal plants belonging to about 44 families are investigated for hepatoprotection and morethan 1.5 million specialists are using the traditional remedial method for health care [3,4]. It is estimated that about 7800 industrialized entities are involved in the manufacture of herbal products [5-6]. A single drug can not be operative for the sever liver diseases [7]. In this manner, a compelling

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definition must be created utilizing native therapeutic plants, with appropriate pharmacological analyses and clinical preliminaries [8]. Polyherbal therapy is said to be a pharmacological principle having the advantage of producing maximum therapeutic compounds with minimum side effects (Aslam). Despite the tremendous strides in modern medicine, there are rare medications that stimulate liver function, offer protection to the liver from damage or help to regenerate hepatic cells. The WHO is engaged to establish definitive guidelines for methodology of clinical research and the appraisal of effectiveness of traditional medicines [9,10]. Therefore in the present review investigate the ameliorative effect of herbal drugs to mitigate hepatotoxicity.

Herbal Medicine and Health

Wellbeing is a state of complete mental, physical and communal opulence and not just the shortfall of illness. Characteristic plant items have been utilized empirically for protective action since ancient times and tendency is emerging today for their increased use. Contribution of the traditional medicine to human health in the 21st Century is of paramount importance. Herbal drugs create a chief share of all the legitimately recognized systems of health in India *viz*. *Ayurveda*, *Yoga, Unani*, *Siddha*, Homeopathy and Naturopathy except Allopathy. Majority of India's population still use these non-allopathic medical systems. In India about 2,500 plants species are known to be useful and more than 6,000 manufacturers produce about 1,500 medicinal preparations from plants [11].

World population in the current growth rate is likely to reach 11.5 billion by the year 2020. Rise in inhabitants, insufficient resources of medications in certain parts of the world, prohibitive cost of treatment for common ailments, side effects of several allopathic medicines in current usage and advancement of protection from at present utilized prescriptions for irresistible ailment have lead to increased prominence on the usages of plant resources as basis of medicines for the wide variety of human ailments. India due to its wide range of geographical, ecological and biological diversities possesses many species that are directly or indirectly cast-off as sources of herbal, allopathic or homeopathic medicines. A significant number of the non-industrial nations practice conventional medication as its primary wellspring of medical services, which is ordinarily of plant beginning [12,13]. Today, almost 88% of the worldwide populaces switch to plant based drug as their first line of safeguard for supporting wellbeing and fighting diseases [14, 15]. Considerable research on pharmacotherapy has been carried out on Indian medicinal plants, which are also rich sources of antioxidants.

Hepatocellular diseases

Today, human beings are exposed on a daily basis to certain foreign chemicals collectively referred to as xenobiotics which are causing serious health problems. Many of these are relatively insoluble in water, soluble in fats and they incline to screen into the hydrocarbon layer of membranes and the fat globules of adipose cells rather than being excreted in the urine. Thus they accumulate in the body

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with deleterious consequences. Liver is an essential organ that assumes a significant part in digestion and discharge of toxicants from the body. Liver toxicity is a significant medical issue that challenges medical care experts as well as the drug business and medication administrative organizations. Hepatic injury caused by various drugs (anti-biotics, antituberculosis drugs, chemotherapeutic agents, carbon tetrachloride, thioacetamide etc.), extreme consumption of alcohol, microbes is well-documented. The liver disorders are worldwide health problem. It is a vital organ for drug metabolism and appears to be profound target site for substances modulating biotransformation. Despite frequent occurrence of liver ailments with great morbidity and mortality, its medical management is currently inadequate and no therapy has been developed which successfully prevent the progression of hepatic diseases. Although newly developed drugs are being used to treat chronic hepatic disorders but they often impose side effects [16,17].

Hepatic damage results in jaundice, fatty liver, cirrhosis, and hepatitis [18-20]. The common causative agents of liver injuries are toxic chemicals, antibiotics, antitubercular drugs, alcohol, hepatitis virus and malarial parasites. Acetaminophen and isoniazid are also inducing hepatotoxicity in human. Isoniazid and rifampicin, the first line drugs used for tuberculosis therapy are associated with hepatotoxicity [21]. Hepatic ailment rate has been accounted for to remain a lot higher in agricultural nations like India (8-30%) compared to that advanced countries (2-3%) [22]. Alcohol-related problems now rank among the world's major public health concerns, not only in most developed countries but also in emerging countries including India. The WHO assesses that there are around 2 billion individuals overall who burn-through cocktails and 76.3 million with diagnosable liquor use issues. Overall, there is a causal relationship between alcohol consumption and around 60 types of diseases and injury. Studies among student population in India have reported the use of alcohol to be 12.7 % in high schools, 32.6 % in universities and 9.3 to 15.1 % among college students. Significant no of deaths/diseases are found in different states of India due to alcoholism [23].

Hepatoprotective plants

Natural plants or plant based medications have been used for the most part by botanist, researcher, and analyst universal for the counteraction and treatment of liver disease. Significant examinations have been completed on ethnomedicinal plants; in any case, least complex few restorative plants have pulled in the interest of researchers, to look at them for a treatment of hepatic change (Table 1.) Clinical exploration in this century has affirmed the viability of a few floras in the management of liver alterations. Subsequently, this audit pays to the information on revealed native plants, which are pervasive for treatment of liver disorders.

Azadirachta indica (Meliaceae)

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Hepatoprotective impact of *Azadirachta indicia* leaves powder was considered in contrast to CCl4 initiated liver diseases. The assessment markers utilized were SGOT, SGPT, ALP, glucose, bilirubin, cholesterol and protein. These biochemical indices were essentially changed because of dose of CCl4, yet the aqueous leaves extract of *Azadirachta indica* fundamentally recuperates all markers to normal levels. Silymarin was utilized as a norm for correlation. This demonstrates generally speaking promising impact against liver issues [24].

Andrographis lineata nees (Acanthaceae)

Hepatoprotective effect of *Andrographis lineata* extracts in CCl_4 induced liver injury in rodents. Animal intoxicated with subcutaneous injection of 50% v/v CCl_4 in liquid paraffin. Biochemical constraints such as SGOT, SGPT, SALP and serum bilirubin were assessed to measure the proper functioning of liver. Histological observation of hepatic tissue supported the biochemical deviations. Extracts activity were also comparable to a standard drug [25].

Aegle marmelos (Rutaceae)

Aegle marmelos leaves which is likewise called as Bilva in antiquated Sanskrit, was utilized as natural medication in the Indian System of medication. The hepatoprotective impact of *Aegle marmelos* in alcohol-induced liver damage was evaluated in rats using vital LFTs biomarker. Singanan study on animal model represented that *Aegle marmelos* leaves have an excellent hepatoprotective efficacy [26].

Cassia roxburghii (Fabaceae/ Leguminosae)

Cassia roxburghii seeds has been used in ethnomedicine for various liver issues for its hepatoprotective activity. The methanolic concentrate of *Cassia roxburghii* turned around the toxicity delivered by carbon tetrachloride in portion subordinate way. The concentrate at the dosages of 250 mg/kg and 500 mg/kg are comparable to the effect produced by Liv-52 against hepatotoxins [27].

Cleome viscosa (Capparidaceae)

The hepatoprotective efficacy of the *Cleome viscosa* Linn extract was evaluated in carbon tetrachloride induced hepatotoxic rats. Plant extract found operational as protection of liver. The hepatoprotective activity of alcoholic extract was comparable to that of silymarin, a standard hepatoprotective drug [28].

Coccinia grandis (Curcubitaceae)

Fruits extract of *Coccinia grandis* Linn evaluated against CCl_4 prompted hepatotoxicity in rats and levels of AST, ALT, ALP, total proteins, total and direct bilirubin were evaluated. At a dose level of 250 mg/kg, the alcoholic extract decreased the LFTs biomarkers which were comparable to that of silymarin revealing its hepatoprotective effect [29].

Cichorium intybus (Asteraceae)

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It is used as anti-hepatotoxic, antiulcerogenic, anti-inflammatory, appetiser, cardiotonic, depurative, diuretic, and contains a variety of bioactive compounds, *like*, inulin, sesquiterpene lactones and coumarins, which plays a key role in medicinal and dietary purposes [30].

Ficus carica (Moraceae)

The methanolic extractile of *Ficus carica* Linn. was evaluated for hepatoprotective activity in CCl_4 induced liver damaged rats. Oral dose of methanolic extract at 500 mg/kg showed a significant defending reflected by depressing the levels of AST, ALT, total serum bilirubin, and malondialdehyde equivalent, an index of lipid peroxidation of the liver [31].

Prostechea michuacana (Orchidaceae)

Prostechea michuacana also studied against carbon tetrachloride induced hepatic ailments in wistar rats. Methanolic extract treated group of animal reduced hepatotoxicity at dose-dependant manner. This hepatoprotective movement is comparable with sylmarin. Other chemical extract like Hexane and chloroform has not shown any specious effect. The discoveries demonstrated that the methanolic concentrate of Prostechea michuacana can be a likely premise of anticipated hepatoprotective methods [32].

Phyllanthus reticulates (Euphorbiaceae)

Phyllanthus reticulatus extract was tested for the hepatoprotective efficacy in animal model alongside carbon tetrachloride induced liver impairment. The animal receiving the extract showed encouraging hepatoprotective efficacy as apparent from noteworthy changes of pentobarbital induced sleeping period, changes the LFTs biomarkers and liver histology of as compared to CCL4 toxicated rats [33].

Rheum emodi (Polygonaceae)

Commonly known as Indian Himalayan Rhubarb, the major constituents are anthraquinones. It is cast-off as a laxative, diuretic to treat kidney stones, gout and jaundice. Its hepatoprotective effect is reported [34]. It contains chrysophanic acid, emodin, glucose rhapatiein, tannin, gallic acid and lignan.

Solanum nigrum (Solanaceae)

The defensive impacts of aqueous concentrate of *Solanum nigrum* against liver toxicity were assessed in CCl4 incited ongoing hepatotoxicity in rodents. The outcomes showed that the treatment of *Solanum nigrum* essentially brought down the CCl4 incited serum levels of hepatic protein markers, superoxide and hydroxyl extremists. *Solanum nigrum* could secure liver against the CCl4 actuated oxidative harm in rodents, and this hepatoprotective impact may be added to its regulation on detoxification catalysts and its cell reinforcement and free extreme forager impacts [35].

Morinda citrifolia (Rubiaceae)

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The hepatoprotective effects of *Morinda citrifolia* juice was evaluated against CCl_4 tempted prolonged hepatic impairment in female Sprague Dawley rats. Serum alkaline ALP, AST, ALT, total cholesterol, triglycerides, LDL and VLDL levels were reduced with the *Morinda citrifolia* therapy. Thus, *Morinda citrifolia* juice appears to defend the hepatocytes from chronic exogenous CCl_4 exposures [36].

Morus alba (Moraceae)

Hepatoprotective effect of *Morus alba* also evaluated against N-Nitrosodiethylamine induced hepatotoxicity in rats. Animal intoxicated with subcutaneous injection of NDEA by dissolving it in milliQ water. Biochemical constraints such as SGOT, SGPT, SALP and serum bilirubin were assessed to measure the proper functioning of liver. LFTs Biomarker regains their normal level after the therapeutic dose of *Morus alba*. Histological observation of hepatic tissue supported the biochemical deviations. Ethanolic extracts of *morus alba* showed the highly effective results which were also comparable to a standard drug silimarin [37].

Leucas lavandulaefolia (Labiatae)

The aerial parts of *Leucas lavandulaefolia* Rees, was tested for hepatoprotective activity against CCl_4 in rats. Ethyl acetate extract of *Leucas lavandulaefolia* has shown significant activity, lowering the LFTs biomarkers in rats intoxicated with Carbon tetrachloride [38].

Vetiveria zizanioides (Poaceae)

Hepatoprotective efficacy of methanolic extract of *Vetiveria zizanioides* Linn root was studied against 20% ethanol tempted hepatic ailmets in rats. Treatment with methanolic extractive of *V. zizanioides* and silymarin significantly prevented the functional, physical, histobiochemical changes induced by ethanol, representing the regaining of liver cells. These results demonstrate that methanolic extract of *V. zizanioides* root possessed the hepatoprotective activity [39].

Wedelia calendulacea (Asteraceae)

Ethanolic concentrate of *Wedelia calendulacea* L. has concentrated against CCl4 instigated intense hepatotoxicity in rodents. The treatment with ethanolic concentrate of *Wedelia calendulacea* showed a portion subordinate decrease in CCl4 initiated raised serum catalyst exercises with equal expansion in complete proteins and bilirubin, demonstrating the concentrate could improve the arrival of ordinary practical status of the liver [40].

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Table 1. List of Hepatprotective Plants

S. No.	Plants	Liver Parameter	Citation
1	*Acathopanax senticosus*	AST, ALP	[41]
2	*Ficus hispida*	GOT, GPT, bilirubin, ALP	[42]
3	*Rhazya stricta*	Pentobarbitone induced sleeping time, GSH, AST, ALT, gamma glutamyl transferase, cholesterol, liver weight	[43]
4	*Cassia fistula*	GOT, GPT, bilirubin, ALP	[44]
5	*Angelica sinensis*	ALT, hepatic nitric oxide synthase activities, GSH, MDA	[45]
6	*Silene aprica*	Morphological and biochemical observations.	[46]
7	*Trianthema portulacastrum*	GOT, GPT, ALP, bilirubin, total Protein	[47]
8	*Bauhinia racemosa*	GOT, GPT, ALP, SOD, CAT, LPO, GSH, bilirubin, total Protein	[48]
9	*Centaurium erythraea*	GPT, GOT, LDH	[49]
10	*Berberis tinctoria*	GOT, GPT, ALP, bilirubin, total protein, lipid peroxidation GSH, SOD, catalase activity	[50]
11	*Zingiber officinale*	ALT, AST, ALP, LDH, SDH	[51]
12	*Moringa oleifera*	ALP, AST, ALT, LPO and TBARS	[52]
13	*Ginkgo biloba*	ALT, AST, tumor necrosis factor alpha in blood, GSH, MDA.	[53]
14	*Aegle Marmelos*	TBARS, GSH, SOD, GPx, CAT,	[54]
15	*Calotropis procera*	GPT, GOT, ALP, biliribuin, cholesterol, HDL, tissue GSH.	[55]

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16	*Raphanus sativus*	Thiobarbituric acid reactive substances, GOT, GPT, GSH, catalase.	[56]
17	*Phyllanthus polyphyllus*	AST, ALT, ALP, total bilirubin, LPO, total protein, SOD, catalase, GPx, GST	[57]
18	*Enicostemma littorale and blume and Eclipta alba*	AST, ALT, ALP,SOD, CAT, LPO and TBARS	[58]

Conclusion

Herbal and conventional botanical products had been used considering historical instances for the remedy of numerous issues and sicknesses. Those natural plants had been mentioned which have been formerly explored through numerous researchers for their hepatoprotective sports. Several medicinal plants show off now no longer simplest hepatoprotective activity, however additionally an extensive variety of anticancer, diuretic, antiarrhythmic, and diverse therapeutic approaches. Hepatoprotective plants are vital for the creation of medication which might be much less costly, have fewer facet consequences, are extra potent, and permit powerful remedy developed for hepatoprotection. Herbal cures are unfastened from facet consequences and toxicity, not like allopathic meds. Studies on hepatoprotective plants will make a contribution to the achievement of the populations needing herbal remedy for hepatotoxicity.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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Development of *in-silico* based Intron Length Polymorphism (ILP) Marker in medicinally important *Catharanthus roseus plant*

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Abstract

Catharanthus roseus (Madagascar periwinkle) is a well-known plant with high medicinal value. Traditional methods of crops or plants improvement like conventional breeding (classical breeding) program have been largely limited by self-compatibility and heterozygosity. Recently, DNA based molecular marker-assisted breeding has increased the speed of breeding program; reduce the manpower as well time to develop elite varieties. Although the development and application of large-scale markers has been reported in *Catharanthus roseus*, but till now, Intron-length polymorphim markers (ILP) was not reported. For the development of intron length polymorphism (ILP) markers, 22,867 EST sequences were retrieved from NCBI database and pre-processed. The overlap sequences were identified and only single and coatings sequences were used in the study. ILP markers were designed by comparing the EST sequences with the available genomes of model plants. Approximately 38 primer pairs were designed for the *C. roseus* from flanked potential intron positions. The BLASTx (EST) analysis of 22,867 express sequence tags suggested that the 55.5% function for ESTs sequence edit them into 2 different functional categories. The developed ILP primers representing the different genic regions of *C. roseus* and will be able to identify polymorphic characters and gene position, diversity analysis and study of transferability.

Keywords- *Catharanthus Roseus*, Intron-Length Polymorphism (ILP), molecular marker, genome, ESTs, PIP database

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INTRODUCTION

The *Catharanthus roseus* is an important medicinal plant in the Apocynaceae family and it is widely used as a source of chemotherapeutic drugs. This family mainly contains herbs and small shrubs. It has smooth marginal leaves, the flowers are found in leaf axils, which are born separately or in pairs on very short stems, and it has another distinctive feature which is the potent milky sap (1).

Catharanthus roseus grows to a height of 20-60 cm, the flowers of this plants are pink, white or rosy-purple. The flowers have a base tube 2.5-3.0 cm long, about 2.0-5.0 cm in diameter, with five petal- like lobed. There are 5 sepals, 2-6 mm long, narrow, usually with pubescent.

Fig1- Picture of *Catharanthus roseus*

Flowering is usually happening during the summer months (March to May months). Very high temperature is not suitable for flowering. Leaves are simply oppositely arranged on the stem, with the entire margin if the leaves, also the plants have an imperceptible and indistinguishable fruit. These Plants are either propagated by seeds or by vegetative methods. Room temperature (25^0C), dark conditions and low water are the suitable conditions for the germination of the seeds (2,3).

The flowers of *Catharanthus roseus* are pollinated by butterflies and moths, this species is self-compatible, although under normal conditions self-pollination is rare. This plant also can be use as ornamental plant in garden and homes across warmer places, or can be grow in glasshouse throughout cold season. *Catharanthus roseus*, better known as the Periwinkle of Madagascar, is native to the island of Madagascar in the Indian Ocean. Madagascar is located on the east coast of South Africa. The Periwinkle is a perennial plant that is very common in tropical and subtropical forests (2, 4).

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Till date several medicinal plants were already identified around the globe. However it was reported that *C. roseus* is one of the most important medicinal plants due to avainlability of more than 200 secondary metabolites. Every part of plant (stem, root, leaf) are highly useful. They are the rich source of Alkaloids (TIAs). In addition to alkaloids, *Catharanthus roseus* produces a wide range of phenolic compounds, including C6C1 compounds such as 2,3-dihydroxybenzoic acid, as well as phenylpropanoids, such as cinnamic acid derivatives, flavonoids. The formation of these compounds in *C. Roseus* is reconsidered, as well as their biosynthesis and regulation of the path. Both types of compounds compete with the biosynthesis of indole alkaloids (4-6).

Introns are very important building blocks of any genomes and scattered throughout the genome. These are the non-coding sequences present in the gene that are transcribed, but removed during the pre-processing. For example, introns make up ~25% of the human genes, respectively (7). In general, introns have little functional significance, although some insertions can affect levels of gene expression. Consequently, introns are more variable than coding sequences.

In plant, fluctuations or polymorphisms can be identified by genetic markers (DNA based marker) with the help of PCR based technologies, which are very useful tools for genetic research (for example, building genetic maps, defining genes or locations for quantitative properties). With the help of DNA markers and mapping population, several genetic maps were already developed in several crops (8, 9-12). On the basis of sequencing technologies and development of new tools, several DNA based markers have been developed, such as microsatellite or simple sequence repeat (SSR), Insilco based markers, single nucleotide polymorphism (SNP) and ILP marker etc. (12-18).

Variation in Intron sequences can also be used to detect the polymorphism. They have been used successfully in mapping research genetics and population genes. It can be easily detected by PCR. To amplify introns by PCR, primers can be designed from flanking exons. This approach is called exon-primed intron-crossing PCR (EPIC-PCR). This approach provided a new method to identify and amplify the DNA sequences. It was reported previously that exon sequences are highly conservative; due to this unique character primers designed from these flanking sequences will be highly use full in several studies.

Because of their unique properties, ILP markers are unique because they are gene specific, co-dominant, hyper-variable, neutral, convenient and reliable. In addition to the sequence tagged sites markers, ILP markers also have transferability ability to amplify adjacent plant species.

To facilitate the direct advancement of ILP markers (13, 17) developed an online database called PIP (Potential Intron Polymorphism) to provide detailed information on various types of markers, indicators and homologous relationships. Despite these advantages, no reports are available till

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date on the uses of ILP markers in medicinal important *C. roseus* plant. The primary objective of the present study was to development of ILP from the publicly available *Catharanthus* ESTs and their Characterization.

Materials and Methods

Molecular markers are most popularly used for estimation of polymorphisms, relatedness & mating system parameters, genotype characterisation in medicinal plants.

Hence, ILP was developed for *Catharanthus roseus*. Therefore, the ILP markers were developed for the same.

Identification of EST sequences

First, the *Catharanthus roseus* EST sequences were retrieved in FASTA format from the NCBI (https://www.ncbi.nlm.nih.gov/) i.e. National Centre for Biotechnology Information advanced science & health by providing access to biomedical & genomic information.

- Open the web browser & enter NCBI in the query, & click on the first link for the NCBI.
- Enter *Catharanthus roseus* in the query box of the page & select Nucleotide from the database, further click on the search button.
- Click on EST from the search result, to select ESTs.
- Go down on the page & click on Send file & select File, then select FASTA in the format & accession in sort by, & click on create file.
- Hence the EST file of the desired EST is developed and downloaded.

Pre-processing of the sequences

The pre-processing was done for the FASTA sequences retrieved from the web, with the help of the web server of the software named as CAP3 (http://doua.prabi.fr/software/cap3) to identify the unique EST sequences.

The Cap3 algorithm computes overlaps between sequences & then joins the reads in decreasing order of overlaps to form Contigs.

- Opened http://doua.prabi.fr/software/cap3 & entered the sequences from the FASTA file retrieved from NCBI Database.
- "Cap3 gave the two files after the pre-processing, that is Contigs & the Single tone sequences & therefore, the further processes were done separately for these."

Selection of candidate EST sequences

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Basic Local Alignment Search Tool (BLAST; https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to identify the specific functions of Non-redundant ILP containing EST sequences. On the basis of blast hit, the homologous genomic regions were identified. BLAST searches were performed to provide complete coverage across the genome sequence.

- Open https://blast.ncbi.nlm.nih.gov/Blast.cgi, & enter the sequences from the file.
- Submit the sequences, by setting the desired parameters.
- Among the various hits, selected the one with the most query coverage, identity & highest scores.

Data Analysis

The data collected from the CAP3 & BLAST were than analysed fir the identification and the characterization of ILP.

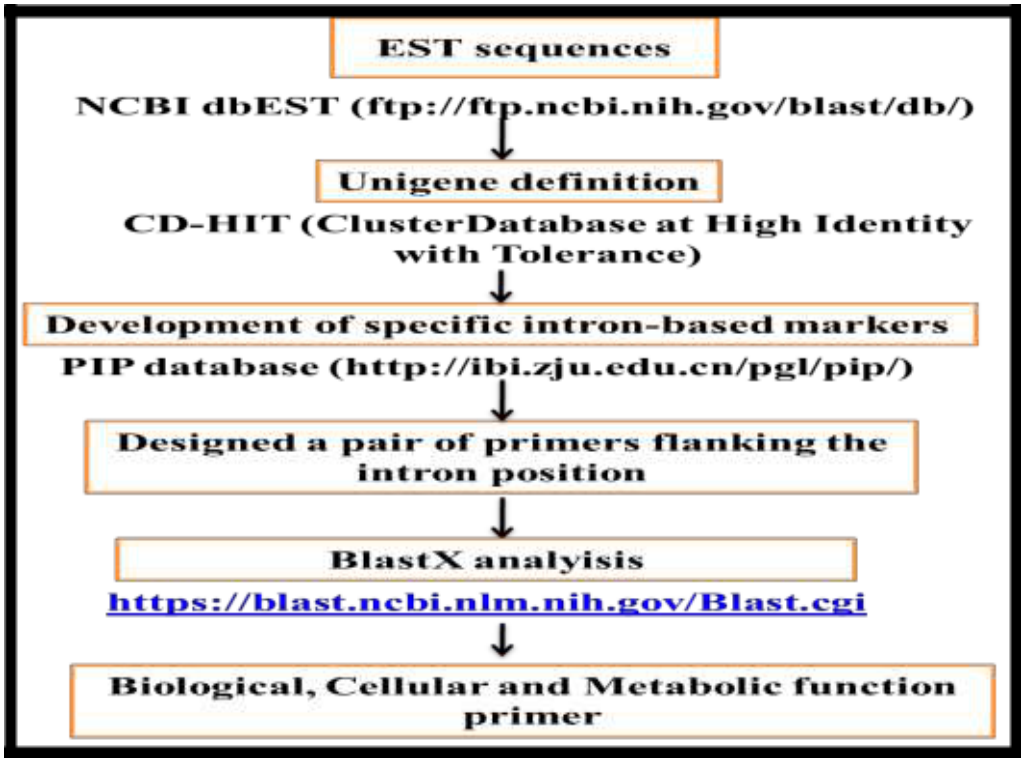

Fig 2- Flow Chart of Methodology

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Results and Discussion

EST assembly

We downloaded a set of 22,867 ESTs from *Catharanthus roseus* EST-database available at NCBI: https://www. ncbi.nlm.nih.gov/.

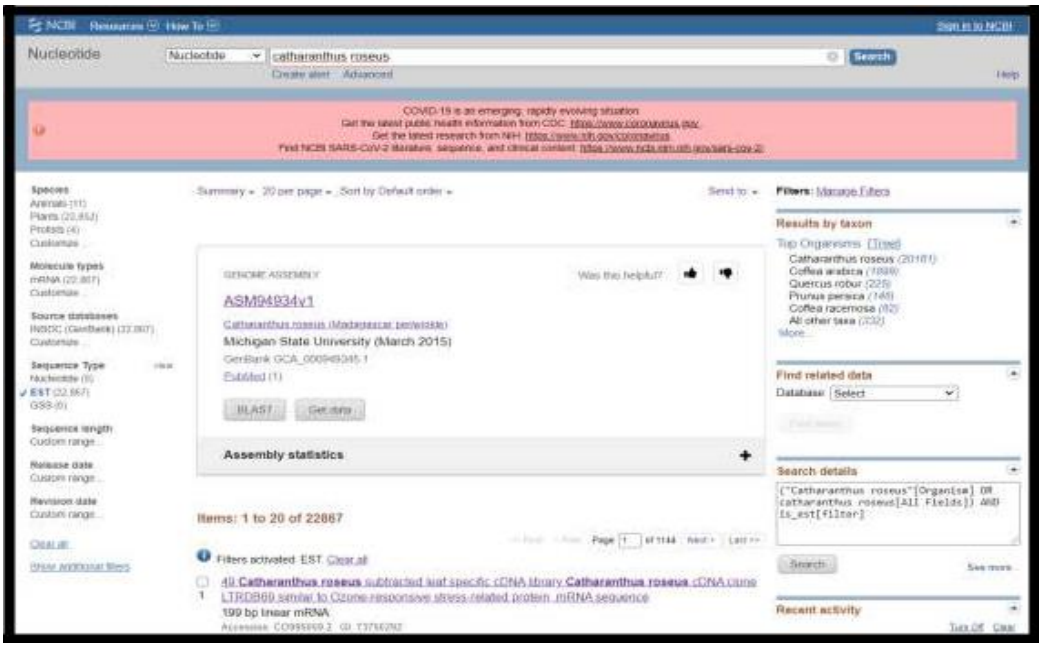
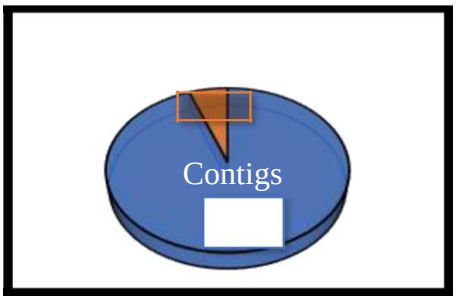


Fig 3: EST Sequences of *Catharanthus roseus* retrieving from NCBI Database (17-07-20)

Pre-processing of sequences

EST sequences of *Catharanthus roseus* were downloaded from ftp://ftp.ncbi. nih.gov/blast/db/. Approximately 22,867 sequences were pre-processed to remove the overlapping sequences. Around 18,992 singletone and 1127 contings were identified and selected by CAP3 software (12, 19). Further these sequences were used to identify SSR containing sequences.



Singletone

Fig 4 - EST sequences of *C.roseus*

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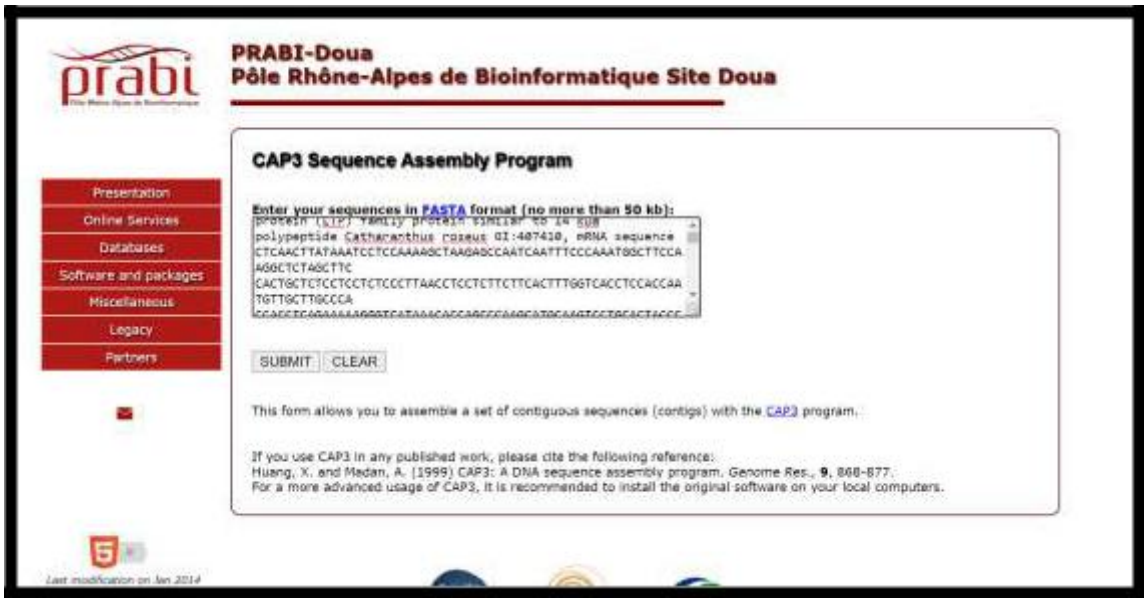

Fig 5- Prabi CAP3 (17-07-20)

ILP mining and primer designing

Unique sequences were processed for developing ILP primers flanking introns using *Catharanthus roseus* singleton genomic sequences. PIP identifies exon intron boundaries and predicts suitable primers flanking intronic regions (Table 1).

Characterization of Primers

Developed ILP primer pairs were characterized by using BLASTX searched and analysed. A cut-off bit score of GC % content above 50% and an E-value of 1e-05 were considered optimum for BLASTX analysis.

The supposed functions of the ILP markers are assigned to perform the BLASTX search for the corresponding markers that contain the EST strings in the NCBI database(http://blast.ncbi.nlm.nih.gov/Blast.cgi) with standard search parameters.

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Table 1 List of primers and their amplification characteristics

S.No.	ID	Tm (˚C)	GC%	Forward primer	Reverse Primer
1	AT4G37930	58	39	GACAACCACTTAGTTTTGGTGAA	CCAGGAACAGTGTTTTTGTTAGC
2	AT1G23490	60	55	TCTCCAGAAGTGGCACAGGT	TCTTCCCAATGCAATGAATG
3	AT5G12250	57	33	ATTTGCTTCAGGACTCTTAAACTT	AATTGAGCTGACCAGGGAAC
4	AT5G54680	60	43	GAGGATTCATGTTCTTCAGTTGC	AAAGAACTTAAAGCTGAGAAAAAC
5	AT2G36580	58	43	GCAATTAAAGTCAAGGCATCC	AAGACGAGGGATGACAACAGA
6	AT5G17770	58	39	TCCTTCCTTGAGGATACATCTTT	AGTCAAGGTGAGGAGGTGATT
7	AT5G17770	57	47	AATCACCTCCTCACCTTGACT	TCAGCCATAATGTAGCAAAGTTTA
8	AT4G39910	60	34	GGGTGGCTTTTTAATTTTCATTC	GAAGAACTTTAGTTCCACCGAGA
9	AT1G12240	59	40	TCCTTGGCATGGAATTTTTC	GACAAGAAGGAATTCGCTCAA
10	AT5G25150	62	63	CGGCCGAGGTACAGCACTA	AGCCACCAGCAACAAGAGAT
11	AT1G13950	61	56	CGGCACCATCCGTAAGAAC	ACTTGGCATGACCGTGCT
12	AT2G39390	59	45	CTGACTGTAATTTCGCAGAAGC	GGATGGCTCTGGTGTTCTTC
13	AT1G51160	59	50	ATGAACACCCTTGTCCAGGT	AAGCTCATGTTTGGCTTGCT
14	AT5G16960	59	35	TAAATATCAATGCCCTCTGGAAA	TGAAGAACAAATTTGGCTTTGAT
15	AT1G76630	59	55	GCCGAGGTACCTTCTCTGAA	TGGAGTGTGAAGGCAATTAGG
16	AT2G19790	59	50	ATGATGTCCAGTTCGCACAC	TTGGTGGGAGTTGACAATGA
17	AT2G19790	59	45	TTCATTGTCAACTCCCACCA	CTCGCACCGAACAACAGTG
18	AT4G26900	59	39	TGCACCTGATCTGAAATACTTTG	TTGTACCGTTGACAGTTGGTG
19	AT4G26900	59	61	CCCCCACCAACTGTCAAC	TTACGGGTTTTCGAGACTTCC
20	AT4G26900	60	60	GTCCCCAAGAGGGAAGTCTC	AACCTTGGCAAGCCAGTAGA
21	AT4G35450	60	57	ACTCCTTGCGTCCGTAACC	GGAGAGGTGAAATGTGCTCAG

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Table 2 Characteristics of EST-derived ILPs for *Catharanthus roseus*.

Primer name	Gene Bank accession no.	Expected Product Size (bp)	Query length	E-value	Putative function
AT4G37930	CO995058.1	110	400	2e-56	hypothetical protein EE612_020327 [Oryza sativa]
AT1G23490	DT527671.1	105	88	3e-45	hypothetical protein SETIT_3G198500v2 [Setaria italica]
AT5G25150	EG562736.1	110	204	7e-24	hypotheticalproteinC5167_049086[Papaver somniferum]
AT1G13950	EG562668.1	101	432	9e-78	eukaryotic translation initiation factor 5A-4 [Cannabis sativa]
AT1G76630	EG562602.1	107	384	3e-36	TPRrepeat-containingprotein[Handroanthus impetiginosus]
AT4G26900	EG562578.1	112	572	6e-105	hypothetical protein CISIN_1g018578mg [Citrus sinensis]
AT1G69620	CX119705.1	100	565	9e-58	Select seq ref|XP_004238799.1| 60S ribosomal protein L34 [Solanum lycopersicum]
AT1G62040	EG562485.1	113	505	6e-40	putative microtubule-associated protein [Oryza sativa]
AT5G52660	EG562479.1	100	676	8e-60	protein REVEILLE 6 [Solanum tuberosum]
AT4G17300	EG562465.1	107	657	6e-84	asparagine--tRNA ligase, chloroplastic/mitochondrial [Solanum pennellii]
AT1G76160	EG562441.1	108	446	6e-70	L-ascorbate oxidase homolog [Nicotiana tomentosiformis]
AT5G27850	EG562407.1	109	535	8e-106	putative 60S ribosomal protein L18-1 [Hibiscus syriacus]
AT1G68370	EG562374.1	101	534	1e-116	chaperoneproteindnaJ15-like[Nicotiana tomentosiformis]
AT2G18110	EG562290.1	108	480	2e-29	elongation factor 1-delta 1 [Citrus sinensis]
AT2G36360	EG562271.1	108	421	4e-58	rab9 effector protein with kelch motifs isoform X1 [Helianthus annuus]
AT3G23390	EG562261.1	108	399	3e-51	hypothetical protein F8388_026239 [Cannabis sativa]
AT5G53560	EG562232.1	111	529	1e-43	cytochrome b5 isoform E-like [Solanum tuberosum]
AT4G27960	EG561828.1	111	504	2e-98	putative aminoacyltransferase, E1 ubiquitin-activating enzyme [Lupinus albus]

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Data mining for development of ILP markers

Around 22,867 ESTs sequences of C. roseus were extract from the EST database, which were collected in 20,119 unique sequences (18,992 Contigs and 1127 singletons) and identified 38 ILP primers. In addition, ILP indicators were extracted from the PIP database. Thus, the PIP database served as a potential resource marker for mining - development of new markers; The remaining strings could not be used to design ILP markers, as they were not able to meet the initial design criteria.

As introns are a non-coding DNA sequence in genes that are not functionally important in plant metabolism, although some genes can affect the level of gene expression. In addition, in the genome they are more variable than the coding sequences due to the total selective pressure in intense regions, much less than in exonic regions (13, 17). Although Huang et al. (16) showed that ILP markers are more versatile than other markers, but there are very few polymorphic ILP markers. In addition, these markers have fewer crosses in wild relatives compared to ILP markers. Thus, these ILP markers are suitable for characterizing wild relatives.

Functional annotation of ILP

BLASTX analyses of the 2,867 EST sequence took on almost many defined functions for ILP markers, and some had nothing in common with previously sequenced genes. The function-based ILP markers were grouped into five main categories with defined function (55.5%) (Figure 2). The largest category (37.2%) contained EST sequences with hypothetical /undeclared/ putative functions. The second largest class (22.6%) included the photosynthesis gene. The stress-related gene (12.5%) ranked third, followed by defence (11.2%), followed by secondary metabolism (8.1%), transformation factors (6.1) and primary metabolism (2, 3%) (Figure 2). *Catharanthus roseus* is a potentially abiotic stress-tolerant plant, particularly for drought salinity, studying EST sequences with hypothetical / undeclared / putative functions (37.2%) and with those that had no resemblance to previously sequenced genes (45, 5%) can introduce new details of stress tolerance mechanisms.

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Functional Classification

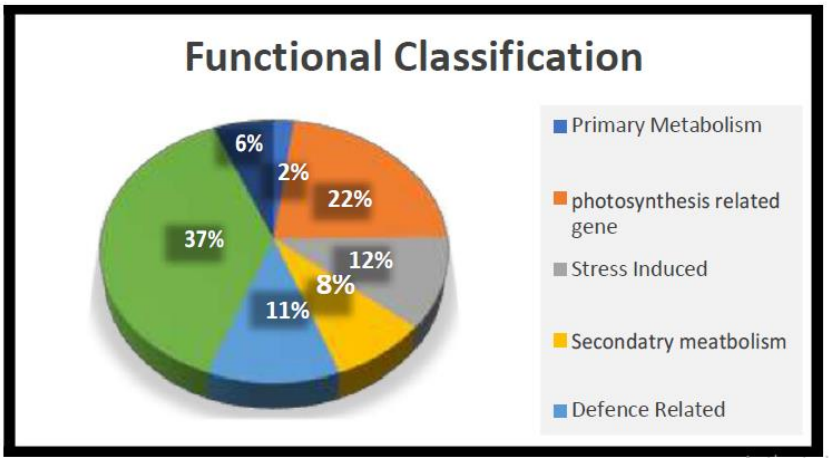

Fig 6- Functional classification of ILP markers containing ESTs/genes. The unique genes were grouped into seven functional groups.

Biological and Molecular Function:

The putative function of the ESTs was attributed to the BLASTx analysis to classify them in two groups based on homology: (a) biological and (b) molecular similar to previous study (12). The significant value of this work is that ILP have shown homology as a target function, demonstrating a good approach for using these ILP sites as a molecular marker to saturate primary and secondary metabolic pathways in plants (13). The polymorphism analysis and transferability of the ILP markers showed the value of the developed indicators. The potency of the ILP developed between species provides a good opportunity to study unknown medicinal plants. The high affinity for these initial pairs, polymorphism and transferability suggests that the markers developed in this study are very useful in auxiliary marker selection, genetic diversity studies, link mapping and comparative analysis. The work presented here complements efforts to develop a well-matured molecular map of *C. roseus*. Recently developed ILPs are informational studies and are a valuable source of gene-based ILP markers.

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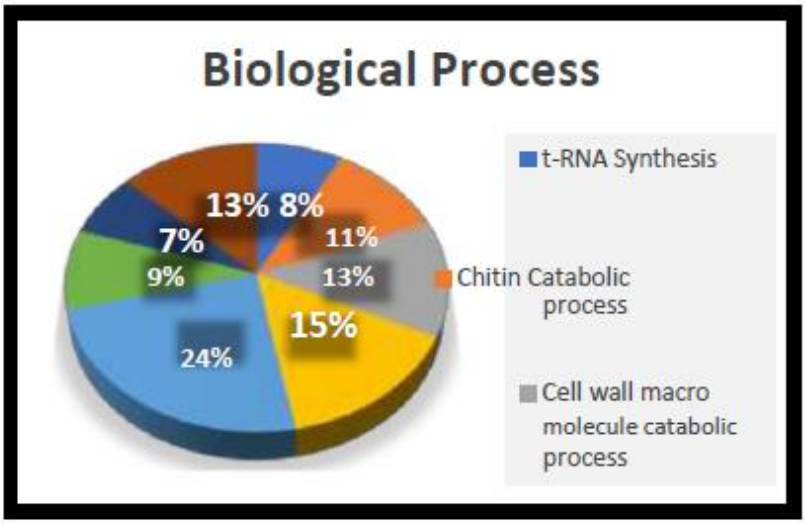

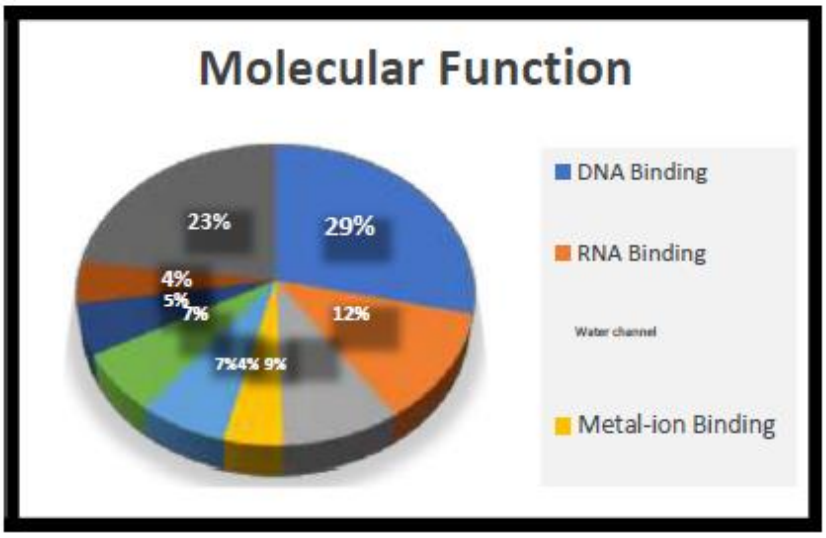

Fig 7. Classification of EST-ILPs on the basis of significant match with putative proteins (A) Biological putative proteins and (B)Molecular Function proteins.

Conclusion

Catharanthus roseus, commonly known as "periwinkle (evergreen)", is a highly studied plant due to its medicinal properties. The secondary metabolites produced by this plant are antileukemic (vincristine, vinblastine) and antihypertensive (ajmalicin

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and serpentine) (20). However, extremely low yields prevent the widespread use of these alkaloids for therapeutic purposes. Molecular marker-based techniques were used to map high-yielding varieties. Currently, ILPs are evolving as a powerful class of molecular markers due to their availability, hyper variability, high availability analysis, high polymorphism, portability compared to other relevant availability indicators. Currently, ILP markers are used in the selection high-yielding varieties, molecular mapping and analysis of quantitative properties. Apart of these unique properties the development of ILP markers are also required lower cost, less time and highly informative (13,17). Although the development of ILP markers has been reported in some plant species (13,17) but in Catharanthus, this is the first report of development of ILP primers. During this study, 38 primers were developed and characterized.

The ILP primers developed from *C. roseus*, can be used for everyone to characterize the identification of a *Catharanthus* species and can be used to characterize and identification of *C. roseus* genotype, genetic diversity, genetic improvement and molecular DNA deformation test of *Catharanthus* species. In addition, the high level of transmissibility of their cross-breed species will increase our understanding of the penetration of genes, evolutionary relationships, among wild relatives of *Catharanthus* species.

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Evolutionary relatedness of human pathogenic bacteria based on conserved and structural gene sequences

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Abstract

Phylogenetic tree is a visual representation of relations between organisms, species and gene sequences. It is a study to represent evolutionary history of living organism using tree like figure. Our indigenous environment contains pathogens which severely affect the host species. Most preferable host is a human body, as it provides nutrients, warm and moist environment which helps pathogens to survive. A molecular characterization was used to point out the pathogenic bacteria at the molecular level without any disturbance of environmental or physical conditions. Molecular characterization started with retrieval of nucleotide sequences of eleven pathogenic bacteria on the basis of 16S rRNA and DNA gyrase gene from NCBI in FASTA format and sequence were aligned by CLUSTALW and tree was constructed by MEGA software. Phylogenetic analysis of eleven pathogenic bacteria on the basis of 16S rRNA and DNA gyrase gene has contributed to illustrate the closely and distantly relatedness of disease causing bacteria and comparison of the both phylogenies interpreted that positions occupied by pathogenic bacteria are different in both the evolutionary trees. As stated by many researchers phylogenetic methods are reliable technique for identification.

Keywords: Phylogenetic tree, Pathogenic bacteria, Molecular characterization, MEGA, 16S rRNA, DNA gyrase.

Introduction

As in the period of Charles Darwin, it is said that about remembering the structure of phylogeny whether it is for individual microorganism or for group of microorganism with their respective species is important (the 16S rRNA sequences convert to branching sequences to represent there evolutionary history). Unluckily, it's very complex form of sequence analysis. Charles Darwin has given two postulates for biological evolution or growth. (1) Innovative evolution occurs by the time and- (2) Dissimilarity in evolution represented by individual according to time. The term 'phylogeny' is defined as a history of an organism which shows evolution. The structure represents evolutionary relationship

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among various organisms called as phylogenetic tree (Morrison 2010). Investigation and test of evolution can be identifying by phylogenetic tree. Phylogenetic tree represents two types of trees *viz.* rooted or un-rooted tree. The phylogeny describes group evolution or group changes in organism. The phylogenetic tree represents phenotypic and genotypic characters (Stephen et al. 2013). Comparative phylogeny method or phylogenetic comparative method (PCMs) permit us to understand about the organismal evolutionary relationship with their common ancestor and another approach is given that it shows diversification or variation, it represents both evolutionary relationship and diversification at same time. Phylogenetics is the surrounding to understand the biodiversity. Phylogenetic comparative method contains a company of numerical data methods for representation of the information which is the combination of two statistics: First relation of species on the basis of their gene and second based on the peer or current characters. Some PCMs help to find history with some geological record or fossils. PCMs are the method of separate set in phylogenetics (Cornwell and Nakagawa 2017). Construction of phylogenetic tree can be enabling by various software. Tree can be constructed by 'neighbour joining method' who represents closely or distantly relationship with peer organism and other method is 'maximum likelihood method' which represents the likelihood or similarity and dissimilarity in characters and functions with the peer organism (Talib et al 2016). In case of pathogen it is beneficial and interesting to know about the disease evolution at genotypic and phenotypic level. It justify about the past and present of disease. The word 'pathogen' is originated from Greek word '*patho*' means ''disease'' and *'genes'* means ''born of 'derived in 1880. Pathogens can be virus and bacteria which cause infection or disease to their host, basically pathogens are an infectious causing agent. Pathogen can be classified on the basis of morphological resemblance (site of infection, way, their host) or resemblance of disease (symptoms, cure process) (Morrison 2010). Pathogenicity is the job of microorganism to cause infection in host's body. The human body is a compound and develop structure. Human body consist of total 1013 cells and approx 1014 normal flora or microorganism which live on surface of host (Alberts et al. 2002). Pathogenic bacteria can enter into human body by different source of transmission. The bacterium reflects it individual pathogenicity. Bacteria are classified into three classes on the basis of their disease causing ability – Primary pathogens are those cause disease to every individual (e.g., *Shigella spp.* isolated from

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human and animal faeces). Opportunistic pathogens are cause disease to host with immunodeficiency (e.g., *Escherichia coli* isolated from urine from the host who is suffering from urinary tract infection). Attenuated bacteria are those are converted to vaccines to resist the disease (e.g., Tuberculosis –*BCG* live attenuated bacteria *Mycobacterium bovis)* (Peterson 1996). Pathogenic bacteria which cause foodborne illness, pneumonia, gastroenteritis, diarrhoea are *Salmonella enterica, Shigella sonnei, Vibrio cholerae, E.coli, Legionella pneumophila, Yersinia enterocolitica, Staphylococcus hominis, Streptococcus pneumoniae*, *mycobacterium tuberculosis, campylobacter jejuni* and *Chlamydia pneumonia*. The identification of pathogenic bacteria is important in many aspects, benefits to doctors to treat their patients accurately and on the basis of research aspects we are able to know about their morphology, characters, phylogeny, structure, evolutionary changes, relationships, disease, symptoms, diagnosis, benefits or loss (Pandey et al.2019). First approach to pathogenic bacteria identification – Close observation is important when the infection is communicated, comprehensive. In USA (Active Bacterial Core Surveillance) is set up in 1995 and in EU (European Centre for Disease Prevention and Control) in 1996, WHO also reported the surveillance in 2014 across the world (World Health Organization (WHO). Second approach is to identify or detect about its diagnosis and to check that "is it bacterial infection or any other infection." Third approach is to check that bacterial strain is susceptible or resistant. Fourth approach is classification and identification method. Classification - Making a list of organism on the basis of characteristics and placing them with their related species. Identification – To identify the unknown bacteria on the basis of character sticks. Identification techniques take place in two aspects: first Biochemical characterization and second Molecular characterization. The biochemical characterization gives morphological information. In clinical microbiology labs the traditional techniques are used to detect most of the sample of pathogenic bacteria strain. Firstly the isolation of bacteria is done then we move to further process of biochemical tests. The various biochemical tests are performed: IMViC test is considered a group test for an individual bacterium. A coli form group test which identify in such a way e.g. aerobic or anaerobic, facultative, gram positive or negative, rod or cylindrical shape. *Yersinia enterocolitica, Staphylococcus aureus, Klebsiella pneumonia* has been detected by this test; agar-based media is the media which support the growth of pathogenic bacteria. The specific culture is important

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to identify pathogens, (e.g. blood agar), MALDI- TOF MS (matrix assisted laser desorption/ionization – time of flight mass spectrometry) has been done to identify *Bacillus anthracis,* fatty acid profiles test is used because in bacterial cell wall fatty acids are essential components to determine the lipid structure A and lipid structure B, flow cytometry is process which used to define the chemical and physical structure of the cell or particles. The sample containing cell is run in flow cytometry instrument (Picot et al. 2012) Molecular characterization method is based on genome and provides genotypic information. Molecular characterization is measured by DNA sequencing. It is the identification at the molecular level without any interruption of environment, physiological state, development or growth. It represents the serology approach, two check relationship between two unknown bacteria. The molecular methods are: Phylogenetic analysis – Analysis based on 16S rRNA of bacteria. Phylogenetic analysis is done by some software e.g. MEGA –X. Phylogenetic tree construction takes place, nucleic Acid and hybridization techniques – In this detection nucleic acids DNA or RNA which are single stranded are permitted to form hybrid, by interacting with complexes, PCR – (Polymerase chain reaction) it is a universal method, it permit accurate identification of bacteria. First we have to obtain 16S gene sequenced, against the bacterial DNA databases then bacteria is identified, DNA fingerprinting – The process of analysis of DNA to identify individual or species. DNA profiling is basically a forensic experiment. (Elijah. et.al 2014), DNA microarrays technique- It is also known as the DNA chip technology. It is a laboratory tool used to detect the expression of thousand genes at the same time (Taub et.al 1983), whole genome sequencing – It is process to describe whole DNA. It is mostly used in medical researches and clinical practices (Gilissen 2014).

Materials and methods

Retrieval of nucleotide sequence of 16S rRNA gene of pathogenic bacteria from NCBI

NCBI is the national centre for Biotechnology information; it is a collective series of databases applicable to biotechnology and biomedicine. Bioinformatics tools are also available in this source. It contains various databases for Genbank for nucleotide sequences, Pubmed for research papers (Stephen 2007). To compare closely or distantly relationship among organism, to check diversification, evolution among organism, the

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nucleotide sequence need to be downloaded. The procedure to download nucleotide sequence is as follow:

- Go to home page of NCBI.
- Then nucleotide as a resource was selected by clicking on 'all database.'
- Then typed the name of pathogenic strain along with the gene 16S rRNA.
- There after various results got visible on the site.
- After clicking on FASTA, 16S rRNA sequence became visible.
- Then sequences were copied successfully along with accession number and saved in a notepad file (Adhikari et al. 2015).

Retrieval of nucleotide sequence of DNA gyrase gene of pathogenic bacteria from NCBI

DNA gyrase can be known as simply gyrase also. DNA gyrase is a tetramer enzyme contain 2gyr A ('A') and 2gyr B ('B') subunit. Gyr B is a housekeeping gene and plays a chief role in replication. DNA gyrase is an enzyme of topoisomerase class and subclass of topoisomerase type 2. The phylogenetic relationship cannot justify on the basis of 16S rRNA only, that's why comparison is essential. The DNA gyrase gene sequence was also retrieved from NCBI. The procedure to download nucleotide sequence is as follow:

- Go to home page of NCBI.
- Then nucleotide as a resource was selected by clicking on 'all database.'
- Then typed the name of pathogenic strain along with the gene gyrase.
- There after various results got visible on the site.
- After clicking on FASTA, gyrase sequence became visible.
- Then sequences were copied successfully along with accession number and saved in a notepad file (Adhikari et al. 2015).

Alignment of sequences by CLUSTALW

Widely used system is a CLUSTAL W for aligning any number of sequences. The method of aligning multiple sequences CLUSTALW has progressive alignment tool. In this tool closely related sequences or similar sequences are those, that contain best score are aligned first. Then progressively the distant or dissimilar sequences are aligned only when global alignment is obtained. CLUSTAL W performs very well and clean task. The algorithms start calculating a distant matrix between the pair of sequence based on pair

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wise sequence alignment. The scores are calculated using pair wise alignment framework for DNA and protein sequences The method for alignment is: installed a MEGA software in window then open the mega software, click on alignment, select the alignment explorer and create new alignment, then click on insert sequence from file and upload your sequences, click on ClustalW tool a multiple sequence alignment tool associate with the software, by considering all defaults parameters click on OK, click on save session by giving name on file (Higgins et al. 1994).

Construction of phylogenetic tree by MEGA 10 (MEGA X) Software

MEGA software is also known as molecular evolutionary genetic analysis developed by Penn State University. It is used for conducting statistical analysis for molecular evolution and for constructing a phylogenetic tree. It includes many sophisticated tool in which CLUSTAL W is one of them. CLUSTAL W align the sequences in two methods, first is multiple sequence alignment in which more than two sequences are aligned and second one is pair wise sequence alignment in each pair of sequence is aligned on the basis of parameters DNA and protein sequences. After the alignment of sequences; construction of phylogenetic tree takes place. Phylogenetic tree can be constructed by neighbour joining method or distanced based method is created by Sitou and Nei in (1987). This method explains whether microorganism is closely related or distantly related or sharing of the common ancestors. The method for constructing phylogenetic tree is maximum likelihood method or character based method which explains the likelihood in characters, functions with peer microorganisms. Construction of Phylogenetic tree on the basis of 16S rRNA and DNA gyrase is done in steps: first alignment is done in MEGA 10 software with help of align tool insert a sequences saved in file, after the alignment save the file in MEGA format, then click on phylogeny and selected the bootstrap neighbour joining method for construction of phylogenetic tree, the tree appears in another window can be saved in pdf, can be copied and can be saved in MEGA format also (Chenna et al. 2003).

Results

The nucleotide sequence alignment of 16S rRNA sequences

The 16S rRNA gene nucleotide sequences of *E.coli, Campylobacter jejuni, Salmonella enterica, Yersinia enterocolitica, Chlamydia trachomatis, Mycobacterium tuberculosis,*

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Shigella sonnei, Staphylococcus hominis, Streptococcus pneumoniae, Vibrio cholerae, Legionella pneumophila were retrieved from NCBI in FASTA format.

Table 1 – Pathogenic bacteria of group 1st of 16S rRNA gene with their respective accession numbers, base pairs and associate diseases.

S. No.	Pathogenic bacteria of group -1st	Associate disease	Base pair	NCBI Accession no.
1.	Yersinia. enterocolitica NRBC 105693	Yersiniosis	1,468 bp	AB682267.1
2.	E. coli JCM 5491	Urinary tract infection	1,464 bp	AB394752.1
3.	Shigella. sonnei CECT 4887T	Shigellosis	1,530 bp	FR870445.1
4.	Staphylococcus hominis	Skin infection	1,468 bp	L37601.1
5.	Streptococcus pneumoniae MAF911410	Pneumonia	1,416 bp	AB002522.1
6.	Campylobacter. Jejuni MTG14	Foodborne illness	1,145 bp	LN864495.1
7.	V. cholerae TS15-B	Cholera	1,509 bp	LC487865.1

The nucleotide sequences along with their accession number were downloaded and saved in notepad file. Alignment of 16S rRNA sequence has been done by CLUSTALW present in MEGA software by default.

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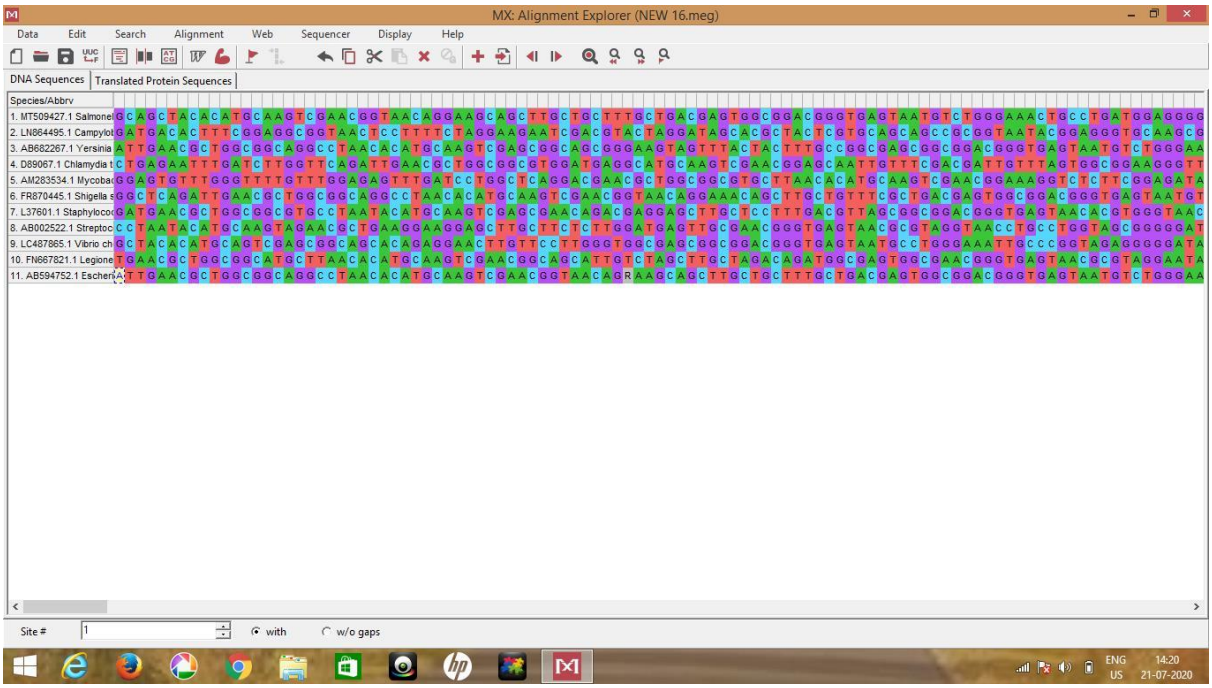

Fig 1- The sequence alignment of 16S rRNA gene sequences of pathogenic bacteria by CLUSTALW.

The nucleotide sequence alignment of DNA gyrase gene sequences

Apart from 16S rRNA gene nucleotide sequences DNA gyrase gene nucleotide sequences of *E.coli, Campylobacter jejuni, Salmonella enterica, Yersinia enterocolitica, Chlamydia trachomatis, Mycobacterium tuberculosis, Shigella sonnei, Staphylococcus hominis, Streptococcus pneumoniae, Vibrio cholerae, Legionella pneumophila* were retrieved from NCBI in FASTA format.

Table 2 – Pathogenic bacteria of group 2nd of 16S rRNA gene with their respective accession numbers, base pairs and associate diseases.

S. NO.	Pathogenic bacteria of group – 2nd	Associate disease	Base pair	NCBI Accession no.
1.	S. enterica subsp. Serovar typhi ST57	Typhoid fever	1,426 bp	MT509427.1
2.	Legionella. pneumophila UCSC22	Legionnaires	1,436 bp	FN667821.1
3.	C. trachomatis HAR13	Conjunctivitis	1,548 bp	D89067.1
4.	M. tuberculosis TB36	Tuberculosis	1,549 bp	AM283534.1

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The nucleotide sequences along with their accession number were downloaded and saved in notepad file. Alignment of DNA gyrase sequences has been done by CLUSTALW present in MEGA software by default.

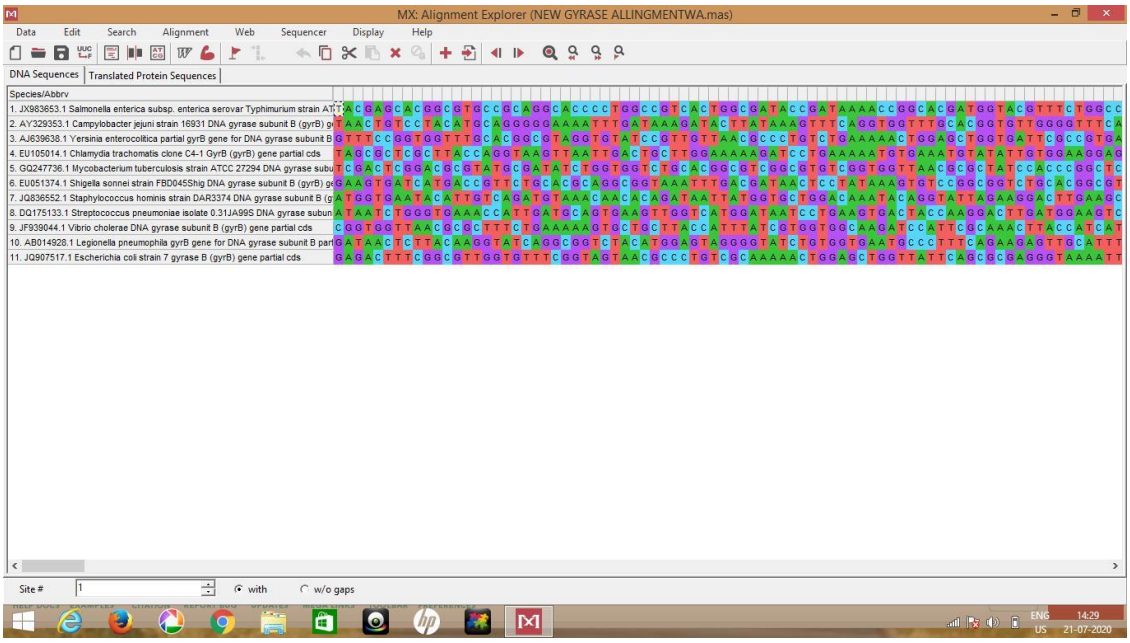

Fig 2- The sequence alignment of DNA gyrase gene sequences of pathogenic bacteria by CLUSTALW.

The phylogenetic tree construction of 16S rRNA gene sequences : The phylogenetic tree was constructed by neighbour joining or distance based method based on 16S rRNA of gene sequences describes the probability value 0.20 and evolutionary relatedness among eleven pathogenic bacteria. The result is given below (fig 3) with the description of different pathogenic bacteria.

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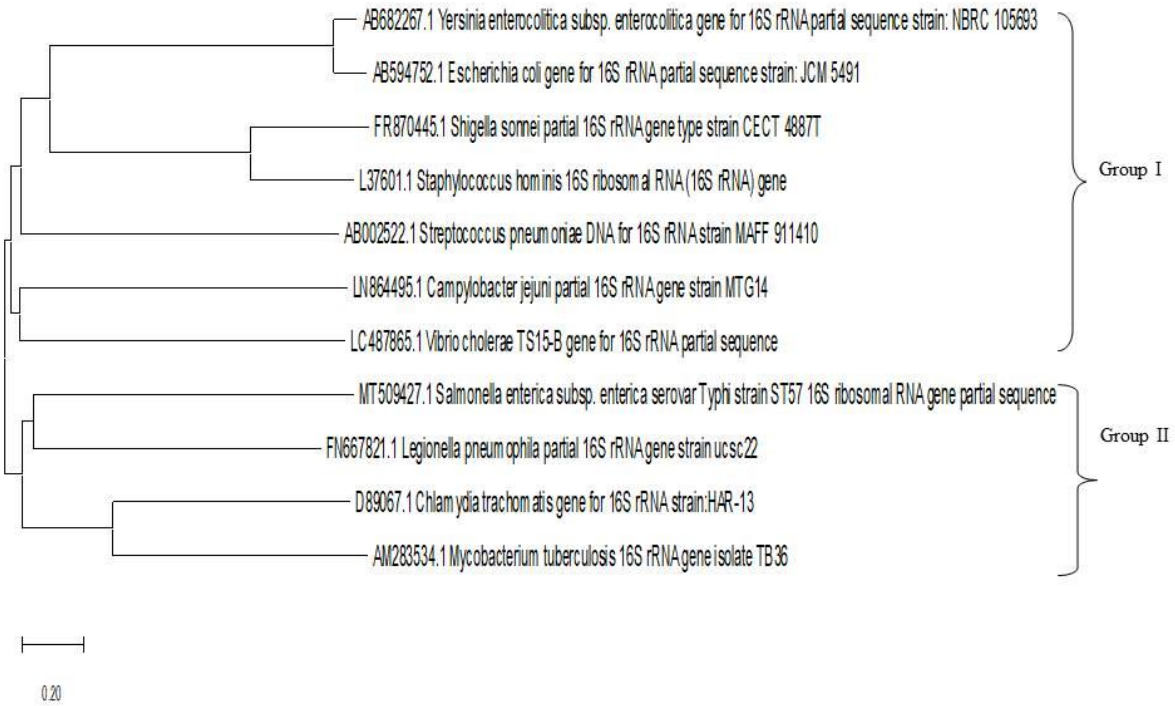

Fig 3 – The phylogeny of pathogenic bacteria based on 16S rRNA gene sequences.

The phylogenetic tree construction of DNA gyrase gene sequences : The phylogenetic tree was again constructed by neighbour joining or distance based method based on DNA gyrase gene sequences describes the probability value 1 and evolutionary relatedness among eleven pathogenic bacteria.

Table 3 –Pathogenic bacteria of group 1st of DNA gyrase gene with their respective accession numbers, base pairs, and associate diseases.

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S. No.	Pathogenic bacteria of group – 1st	Associate disease	Base pair	NCBI Accession no.
1.	Mycobacterium tuberculosis ATCC 27294	Tuberculosis	1,256 bp	GQ247736.1
2.	E. coli 7	Urinary tract infection	1,119 bp	JQ907517.1
3.	Streptococcus. pneumoniae 0.31JA99S	Pneumonia	1,992 bp	DQ175133.1
4.	V. cholerae	Cholera	1,083 bp	JF939044.1
5.	Campylobacter jejuni 16931	Foodborne illness	1,221 bp	AY32935.1
6.	Chlamydia trachomatis C4-1	Conjunctivitis disease in cornea	1,180 bp	EU105014.1

The result given below (fig 4) with the description of different pathogenic bacteria.

Fig 4- The phylogeny of pathogenic bacteria based on DNA gyrase gene sequences.

Table 4 –Pathogenic bacteria of group 2nd of DNA gyrase gene with their respective accession numbers, base pairs and associate diseases.

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S. No.	Pathogenic bacteria of group -2nd	Associate disease	Base pair	NCBI Accession no.
1.	Staphylococcus hominis DAR3374	Skin infection	1,935 bp	JQ836552.1
2.	Legionella pneumophila gy10041.icb	Legionnaires	1,167 bp	AB14928.7
3.	S. enterica subsp. Serovar typhimurium ATCC14028	Typhoid fever	1,037 bp	JX983653.1
4.	Yersinia enterocolitica ATCC 96010T	Yersiniosis	1,038 bp	AJ639638.1
5.	Shigella sonnei FBD045shig	Shigellosis	1,256 bp	EU051374.1

Discussion

Phylogenetic trees are commonly used for optical representation in the life sciences and the most essential optical representation in evolutionary studies. After the victory of phylogenetic tree construction, results clarified that position of bacteria are different in both the phylogenetic trees fig 3 and fig 4. Closely related species represents that they are sharing common ancestors and sharing same genes among their peer groups. The representation of fig 3 clarify that *Yersinia enterocolitica* strain shares the most recent common ancestor with *E.coli* strain, *Shigella sonnei* strain and *Staphylococcus hominis* strain are also sharing most recent common ancestor. The tree representation of fig 4 explains that *Yersinia enterocolitica* strain shares the closely relationship with *Shigella sonnei* strain and same as *Campylobater jejuni* strain is closely related to *Chlamydia trachomatis* strain. Grouping in phylogeny represents the last common ancestor, origin of root. As shown in table 1 the group 1st of pathogenic bacteria which are the *Yersinia enterocolitica* strain, *Escherichia coli* strain, *Shigella sonnei* strain, *Staphylococcus hominis* strain, *Streptococcus pneumoniae* strain, *Campylobacter jejuni* strain, *Vibrio cholerae* strain are sharing same root, somewhat sharing common ancestor with each

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other in phylogenetic tree of 16S rRNA genes and the phylogenetic tree of DNA gyrase gene represents the group 1st group of pathogenic bacteria in table 3 that *Mycobacterium tuberculosis* strain, *Escherichia coli* strain, *Streptococcus pneumoniae* strain, *Vibrio cholerae*, *Campylobacter jejuni* strain, *Chlamydia trachomatis* strain are sharing the distantly related common ancestor with one another. After the comparison of phylogenetic representation of both 16S rRNA and DNA gyrase gene, result interprets that estimation of evolutionary history and relationships cannot be done only on 16S rRNA. Both the tree shows the changes in position of disease causing bacteria (Naushad et al. 2016).

Conclusion

Comparison of phylogenies on the basis of 16S rRNA and DNA gyrase genes sequences provided the details about the evolutionary relationship of disease causing bacteria. Construction of phylogenetic tree is one of the chief techniques of molecular characterization to understand about the evolutionary relationship among different organism. According to the scientists, the phylogenies can explain the disease at genetic level and provides the details about the history, current situation and afterwards of pathogens. According to many researchers phylogenetic methods are reliable for identification of pathogens. The accomplishment has shown that the all eleven pathogenic bacteria have different position in phylogenetic tree of 16S rRNA and DNA gyrase. The pathogenic bacterial strains have acquired the positions according to their gene sharing with the fellow groups and their common ancestors. The future of phylogeny will be the considerable data to preserve its relations with ancient root and prompt biological data through species names and evolution. Pathogens have different distribution; different mode of action, different rate of growth can be understood by phylogeny. This justify different characters at gene and geographic level by the comparison of location of isolated sample to investigate source of spread and molecular methods can be applied to check the evolution, events, spreading of new pathogens. Many researchers thought that comparative methods should do better work for collecting multiple data into a systematic framework. Projects with common aims, like evaluating when and why an ancestry undergoes speciation, are better united than separate. Comparative set of data help us to measure about an evolving genus from one generation to the next. Some comparative methods also assimilate information from earth science documentation especially fossils,

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but also other moderate and periodic events in the globe's history. In anthropology recent techniques developed for phylogenetics have been tried successfully to languages in the exact identical ways as creating species phylogenetic trees grounded on genomes. PCMs are progressively used in fields apart from evolutionary biology such as community ecology, anthropology, linguistics, paleobiology. These methods have becoming powerful recently due to the support of data resources and computational power to construct larger and better phylogenies.

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A comprehensive Review on pandemic virus COVID-19 with reference to Prognosis, Diagnosis & Therapeutics

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Abstract

In last week of December 2019, WHO was informed about unidentified cases of pneumonia in Wuhan, Hubei Province, People's Republic of China. The symptoms were similar to viral pneumonia and after diagnosis and identification by Chinese Health authorities in first week of January 2020, [1] Coronavirus was identified as causative virus particle and named as novel coronavirus pneumonia. In last week of January 2020 following the recommendations of the emergency committee, The World Health Organization (WHO) declared the outbreak constitutes a public health emergency of international concern (PHEIC) [2]. The name 'CoVID-19' is given by World Health Organization (WHO) and Sever Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) by International Committee on Taxonomy of Viruses (ICTV). The current level of knowledge on CoVID-19, world scientists gathered in February 2020 and discussed the critical research problems related to this curtail viral outbreak, to fight back for the current situation and the future as well. But still the exponential graph of this viral disease is growing higher and higher each day [3] [4].

It is highly contagious, circulates in nature due to spillover with a range of factors such as having several natural, intermediate and final host. The reservoir for CoVID-19 is not known yet but the genomic analysis had shown the similarity index is 76%and 96% between CoVID-19 and SARS-CoV-RaTG13 found in bats respectively. So it concluded that it might originated from bats. [5] [6]

Key Words: CoVID-19, SARS-CoV-2, Coronavirus, SARS, Sanatization, WHO.

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Introduction

Coronavirus is a large group of enveloped RNA viruses, spherical or pleomorphic in shape. It carries petal like or club shaped peplomers on their surface. The name 'corona' refers to the fringe of surface projections which surrounds the virus which gives it resembles to the 'solar corona'. They are divided into two groups of **acid labile viruses** (common cold) and acid stable viruses (gastroenteritis). There are many serotypes which are hard to diagnose, detect or culture. The first isolated virus of this family was from common cold case. It is the second most prominent causative agent of common cold cases after Rhinoviruses. [7]

Characteristics and Classification

Coronavirus is an enveloped virus which is spherical in shape. The particle size ranges from 120-160 nm in diameter with helical nucleocapsid which is of 9-11 nm in diameter. It consist of linear, non-segmented, positive sense of 27-32 kb single stranded RNA which is capped and polyadenylate. It is infectious in nature and one of the largest genome among viruses. Projections are of 20nm long which are widely spread. The viral structural proteins include phosphorylated nucleocapsid proteins, membrane protein or glycoproteins proteins that behaves as a matrix proteins which are embedded in the nucleocapsid, and the spikes glycoproteins that makeup the petal shaped peplomers. [7] [8] [9]

It causes common cold as well as SARS (Sever Acute Respiratory Syndrome Coronavirus). It have very high frequency of recombination. All the genres of coronaviruses affect animals, birds and humans. It displays very high frequency of recombination. It is hard to grow in cell cultures. Human infection is uncommon except few who adapted to human conditions. [8]

There are six recognized viruses from corona family that are known to cause human infections; Most of them belongs to Betacorona family except first two mentioned below:

1. Human Corona-229E; 2. Human Corona-NL63; 3. Human Corona-OC43; 4. Human Corona-HKU1; 5. SARS-CoV (Severe Acute Respiratory Syndrome); 5. MERS-CoV (Middle East Respiratory Syndrome); 6. COVID-19.

Some Human Corona Viruses (HCV) like OC43 contains third glycoprotein that causes hemagglutination & has acetyl esterase activity. The novel Coroavirus discovered in 2003 from patients of SARS is in the same group as OC43. [7] [8]

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CLASSIFICATION	
FAMILY	CORONAVIRIDAE
ORDER	NIDOVIRINAE
SUB-FAMILIES	CORONAVIRINAE
	TOROVIRINAE
GENERA	ALPHA CORONA
	BETA CORONA
	GAMA CORONA
	DELTA CORONA

Replication of the virus particle:

A virus particle can only replicate in a living cell so it needs a host which can provide energy and a synthetic machinery (cell organelles) having all required precursors for the expression of viral genome to synthesize viral proteins. The nucleic acid of virus particle carries the 'genetic specificity' to code for all the specific and required macromolecules in an organized manner. The unique feature of viral replication is its interaction with the host cell receptors as the envelope proteins of the virus particle are similar to the host cell protein, after the viral particle gets adsorbed and entered the host cell, it gets disrupted losing its measurable infectivity, this phase of growth cycle is called the eclipse period. After a definite interval of eclipse period rapid accumulation of infectious progeny of virus particle takes place. [7]

Replication of some viruses is not very easy to study as they are hard to culture in laboratory, but some of them can be studied on cell cultures. Human corona virus do not grow well in cell cultures so the details have come from different studies of other viruses which are closely related to this virus particle. [8]

The replication cycle of coronavirus includes distinct ten steps including entry, replication of virus, latency, and shedding. The ten stages of the whole replication process is given below:

1. Reception at host receptors.
2. Entry and uncoating.
3. Endosome formation.
4. Release of genome.

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5. Translation
6. Proteolysis
7. Replication
8. Transcription
9. Translation
10. Assembling of virus particle and release. [7]

The replication cycle of coronavirus takes place in cytoplasm. Virus targets the receptors of the host target cells and gets attached on their surface with the spikes (S) or hemagglutinin (HE) and binds to ACE-2. The receptor for human corona virus in human body cells is said to be the aminopeptidase N, and for SARS virus its angiotensin converting enzymes (ACE-2). The virus particle is then internalized by a mechanism known as absorptive endocytosis. The spike protein and HE protein may be responsible for the fusion of the viral envelope and the cell membrane/transmembrane (Serine protease, TMPRSS2) of the host. The very first step just after uncoating is the translation of polyproteins that encodes for replicase-transcriptase complex to produce a virus specific RNA dependent RNA polymerase. A full length complementary (minus strand) RNA is transcribed by the polymerase enzyme of the virus that serves as a template strand for a nested set of five to seven sub-genomic mRNA. The 5' terminal gene sequence of each mRNA gets translated. The full length genomic RNA copies gets transcribed and each sub-genomic mRNA is translated into a single polypeptide. In corona virus infection polyprotein precursors are not common, although a large polyprotein that get processed to yield the viral RNA polymerase is encoded by the genomic RNA. Neonate genomic RNA molecules interacts with the nucleocapsid protein in cytoplasm of host cell to form helical nucleocapsids. The leader RNA have a preferred binding site for N protein. The rough endoplasmic reticulum and Golgi bodies in areas containing viral glycoproteins releases nucleocapsids buds. After maturation the virions may transported to the periphery of the host cell in the form of vesicles for exit or release. Virions formed in corona infection are apparently not formed by budding through the plasma membrane. In some coronaviruses induced cell fusion is mediated by S protein. Some of the coronaviruses establish persistent infection rather being cytocidal. [10] [11]

Coronavirus generally have a very high tendency of mutation during each round of replication, including the generation of substitution mutations and a high incidence of

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deletion mutations which is a big threat for future. This virus undergoes high frequency of recombination which is very unusual for an RNA virus with a non-segmented genome and most of the time contributes to the evolution of a new virus strain like CoVID-19. [11]

Pathogenesis and Clinical features:

Coronavirus infections in humans usually remain limited to the upper respiratory tract but if severity increases can reach up to renal level. In contrast to SARS outbreak in 2003 was characterized by Serious respiratory illness including pneumonia, dyspnea and progression respiratory failure. Virus can also be detected in several organs, including liver, kidney, small intestine, and stool as well. Generally viruses of this family originates in non-human host mostly in bats, Chinese horseshoes bats are natural reservoir of corona like viruses due to widespread use of wild species for food and traditional medicines promotes the emergence of new viral strains. [7] [11] [12]

According to a retrospective study on clinical course an article published in 'The Lancet', after the completion of incubation period of 14-27 days symptoms may arise or may not be noticeable and within 3 days just after onset of infection the virus pass through the mucosal membranes of nasal passage and larynx, entering the lungs generating initial symptoms like fever, soreness in throat and cough occurs. After 4 to 9 days virus started to spread through lungs which results in breathing problems such as heavy breath, shortness of breath leading to hypoxiya and acute respiratory distres. The virus moves further to the peripheral blood streams resulting sepsis or viremia. The virus further targets the organs which express ACE-2 such as lungs, heart, renal, gastrointestinal tract. Some studies shown that onset of infection the WBCs count was found to be normal or slightly low leading to lymphopenia i.e., infection may affect antibody production in the patient. By the time of 3 weeks the crucial period starts either it leads to recovery or death of the patient. The inflammatory factors associated with disease such as IL-6 are also reported to be increased contributing an aggravation of the disease 7-14 days after infection. It has been also reported that in some infected patients exuberant inflammatory response found which is similar to cytokine release syndrome which may leads to critical and fatal illness. It is accompted that the clinical phase includes viremia, acute pneumonia and recovery. If the patient is not immunocompromised the disease can be recovered in acute phase as when the immunity is low the WBCs gets reduced with increase in

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inflammatory cytokines. It has been also observed that in severe patients development of disseminated intravascular coagulation possibly due to high rate inflammation in vascular wall. [12] [13] [14] [16]

The clinical manifestations and symptoms are quite similar to Rhinoviruses (Common cold) identified by nasal discharge and malaise.The incubation period of coronavirus is of 14 to 27 days, it infects upper respiratory tract majorly generating pneumonia, sever respiratory disease to lung failure. The common clinical features of Coronavirus-2 are Fever, Fatigue, Dry Cough, Anorexia, Myalgia, Dyspnea, Anosmia/ Dysensia (loss of smell and taste), Gastrointestinal pain, Nausea, Diarrhea. Because of the infection alveoli gets filled with fluid and the oxygen-carbon dioxide exchange becomes difficult with cough and breathing problems. The state of sever pneumonia or ARDS (acute respiratory distress syndrome) or lung failure takes place. The patients kept on ventilatory support systems due to ARDS. The death rate is higher among elderly people. [7] [15] [16]

Epidemiology and Transmission:

According to the study the very first coronavirus (MERS-CoV) were transmitted from Camels, SARS CoV from Civet Cat and the reservoir for the novel corona virus (SARS-CoV-2/ COVID-19) is said to be transfred from pangolin but it is not confirmed by any study yet.

Bats are said to be the natural hosts while pangolin and snakes are said to be intermediate hosts. The very first study had shown that it might spread through snakes but later it was proven that no such evidence that snakes behaves as intermediate hosts. In some studies the sequencing shown that there is 96% similarity between COVID-19 and the corona virus found in bat, so it is said that bats the possible source of spread. But recent studies done via macro-genomic sequencing, molecular biological detection have shown that the strain isolated form Pangolin and the strain isolated form human are 99% similar proving that pangolin is the potential intermediate host of SARS-COV2 through the results are not fully elucidate the potential of natural/ intermediate host of SARS-COV2. At present the potential source of infection and made of transmission infected patients only. It’s still not clear that the period of incubation inside the patient is also behaves as a transmission phase of this virus. [15] [16] [17]

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According to the proven epidemiological studies, there are majorly 3 conditions for the wide spread of the virus: 1. **The source of infection**, **route of transmission**, and **susceptibility**.;

2. **There is no exception for SARS COV**. [16] [17]

Route of Transmission

Close contact is the most common way of transmission. Apart from that aerosol, surface contact can also be reasons of transmission. Genetic samples were detected in different tissues of gastrointestinal tract, conjunctivital secretions, etc. The reported latency period of SARS-CoV is 14 days. If we look from median point of view COVID-19 have a shorter incubation period than that of SARS and MERS i.e., 24 days maximum period which may increase the risk of transmission. It has also been proven the progression of disease is faster in old age people than that of younger ones. [17] [18]

Infectivity Period

Transmission of the virus occurs prior to the development of symptoms and throughout the course of illness. The viral RNA levels are higher on the time of setting symptoms, so it shows that patients are more tend to spread infection in earlier state of infection than that in later stages. The viral shedding depends upon the severity of illness. In a study the median duration of viral particle shedding from the upper respiratory tract was of 24 days minimum and maximum of 42 days. Risk of transmission grows when proper sanitation and personal protective equipment are not in use. The chances of spread of infection is higher in case of gathering, face to face communication and conversations, contaminated food consumption, gestures exchanged with any infected person, coming in contact with any contaminated surface, etc. [18]

Diagnosis:

The general diagnosis of a suspect of CoVid-19 is to observe the symptoms such as high fever, cold, cough, sneezing, sore throat. However some of the cases reported not to show such symptoms prominently i. e., asymptomatic condition so, the molecular level tests are the best way to detect a positive case. There are 41 regulatory authorized diagnostic tests available till date. The diagnostic test types or scientific technology used for detection are PCR, next gen sequencing (NGS) and isothermal amplification. According to the current information viral nucleic acid detection is the most standard non-invasive diagnosis available for Covid-19. The detection through these methods are highly specific and less

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sensitive, so the false negative are prominent and the time for testing can be longer as well. A report says that for the rapid detection of the virus, Zhang F of MIT University under Sherlock Technologies developed a test paper which can detect the presence of virus in one hour. It is under clinical verification. RT PCR is the most prominent method for detection in which the nasopharyngeal secretions are used to detect RNA extractions, RNA extracted from the deactivated virus particles is further purified and transcribed by RT PCR to synthesize complimentary DNA. A positive patient's sample cross the threshold line within 40 cycles. [19] [20]

A wide range of different tests are availablein Serology. This should be indicated after onsetting of symptomsin second week probably with definite specificityand variable sensitivity. The serological tests required validation of experts as well.The lab tests for CoV-2 are complete blood count, D-dimer, lactic dehydrogenase (LDH),clotting tests,C-reactive protein (CRP), ferritin, and procalcitonin. All of these tests can identify the risk of disease with severity, thromboembolic complications, myocardial damage, and/or worse prognosis. Imaging tests such as CT scan is highly recommended todiagnose, especially when there are clinical symptoms but other tests showing negative results. [63]

Variants of Concern (VOC):

Researches have shown that this virus is mutating very fast. Multiple variants of CoV-2 are circulating globally. There are more than 70 new strains reported in which 3 are declared as variants of concern (VOC). On the basis of genome sequencing data of different samples collected from different states of the country, more than 700 cases were reported affected with the new strains.

New variants recently reported in India are **B.1.1.7** (reported in US in December 2020, initially detected in UK in September 2020), **B.1:351**(earlier reported in US at end of January 2021, initially detected in South Africa), **P.1** (earlier detected in US in January 2021). There are two more variants **E484Q** and **L452**were also found in some parts of India. [62]

Reasons of mutation:

RNA viruses are sensitive towards mutation. It changes gradually according to the geographic separations. The very first mutant B.1.1.7 was reported in UK in September 2020 responsible for 60% of new CoVid cases. It is prominent as a mutant in many other countries including India. This variant is more contagious and more lethal. This virus is

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affecting the younger and the children both. Studies says if it will circulate more there are chances of further mutations which can turn this virus into more lethal and contagious and its worrisome that similar changes are arising in the spike protein of the virus independently some mutation results in no change but some mutation alters the behaviour of the virus which results in different spike proteins and different key areas with changed attributes.

According to different studies, any mutation occurs at amino acid residues from 319 to 541, especially between 438-506 may significantly impact the infectiousness, transmissibility, severity, and its immunity evading potential. [62]

Current Treatment and Ongoing Research:

During the outbreak of this pandemic in 2019 and 2020 there was no clinically proven and directly effective antiviral therapy available for SARS-COV 2 but with the blessing of science and the hard work of scientists we are able to fight back this pandemic viral disease.

Vaccine Research:

Vaccine researches are still in process. Researchers are working designing vaccine by using DNA, RNA, inactivated virus particle, live attenuated virus, non-replicative virus, protein subunits, replicating viral vectors, viruses like particle (VLP). There are more than 50 vaccines under development or in pre-clinical and clinical trials. [21] [22]

There are many vaccines which are already out for clinical trials and got approved by the health ministries of different governing bodies. The very first vaccine dosed a human was in USA named as‘mRNA-1273’ produced by Moderna Company which was designed from synthetic mRNA to induce an immune response to produce antibodies against SARS-COV-2. The other vaccine being developed in China namely $Ad_{s\text{-}n}CoV$ by CanSino Bio with non-replicating viral vectors to deliver antigens to express the SARS-CoV-2 spike protein. The third vaccine is proposed in UK by the scientists of University of Oxford which was also developed from non-replicating viral vector to deliver RNA into cells. China proposed another vaccine namely LV-SMENNP-DC through scientists of Shenchen Geno-Immune Medical Institute. This vaccine uses a lentiviral vector to deliver CoVid-19 minigenes to modify dendritic cells and activate T-cells. Another vaccine was tested by a research group in Netherlands namely BCG vaccine repurposing

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to fight against CoV-2, this same vaccine was proposed in Australia as well by Murdoch Children's Research Institute for CoVid-19 patients. All of these vaccines were mostly used in Phase I, II and III. [23] [24]

Recently one vaccine has approved by the Ministry of Health of the Russia Federation in 2020. This vaccine 'Sputnik V formerly known as Gam-COVID-Vac is developed by Gamaleya Research Institute in Moscow. Many experts have raised concern about its safety and efficacy. [25] This vaccine is made up of genetically modified cold virus which will deliver small fragment of coronavirus to human body in terms of priming the immune system to fight with the coronavirus. The jab uses two different version of vaccine using different vectors both of the vectors target the spike protein. The idea behind is to use two different formulas which will boost the immune system even more which may provide long lasting protection. It is 92% effective against CoV-2. This vaccine is under approval for vaccination drive in India but its efficacy is limited against the new variants of this virus.

In India there are two vaccines approved by health ministry in 2021 Covaxin and Covishield which are said to be highly effective.

Covaxin is an inactivated vaccine which is developed by the "Bharat Biotec Ltd."an Indian pharmaceutical company from a strain of coronavirus isolated by the National institute of Virology. This vaccine uses inactivated virus (killed virus) which is recognized by the immune system and leads to the production of antibodies. Covaxin is given in two doses four weeks apart.

The Oxford AstraZeneca (Covishield) vaccine is designed by the "Serum Institute of India" (world's largest vaccine manufacturer). This vaccine is made up of a weakened version of a virus causing common cold (adenovirus). The jab is administrated between 4 to 12 weeks apart which is designed by "Pfizer BioNTech".

There are other vaccines as well which are under clinical trials and waiting for further approval ZyCoV-Di by "Zydus-Cadila", HGCO19 by Genova in collaboration with Seattle based private pharma company HDT Biotech Corp., NovaVax. [61]

The below table is showing the comparative performance of the available vaccines in India against CoV-2 and its new variants: (Source: Dr Vipin Vashishtha)

S.No.	**Vaccine**	**Original' (symptomatic)**	**B.1.1.7 (symptomatic)**	**B.1.351 (symptomatic)**	**P.1 (symptomatic)**

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1.	AstraZeneca (Covishield)	81.50%	70.40%	10.40%	NA
2.	Novavax	89%	85.60%	49-60%	NA
3.	J&J (Janssen)	72%	NA	57-64%	68%
4.	Pfizer	95%	Neutralisation decreased 2x	Neutralisation decreased 6.5x	Neutralisation decreased 6.7x
5.	Moderna	94.10%	Neutralisation decreased 1.8x	Neutralisation decreased 8.5x	Neutralisation decreased 4.5x

Drugs and Therepies:

Drugs and therapies in use to combat CoVid-19 around the world are selective namelyArbidol, Cermostatmesylate, Hydroxy chloroquine, Lopinavir, Darunavir, Ribavirin, Remdesivir, Flavipiravin, Blood thinners and Plasma therapy. [26]

Chloroquine and Hydroxychloroquine

This drug is commonly used for the malaria treatment and chronic inflammatory diseases. Studies reported that it blocks the entry of the virus into cells. It inhibits the glycosylation of host receptors, proteolytic processing and acidification of the endosomes. It was in use for the treatment of CoV-2 widely, it inhibits the virus with half maximal effective concentration [EC_{50}] in the low micro molar range. Dosing has consisted of 500mg orally once or twice daily but according to some studies dosing recommendation is 400mg orally once a day or 200mg orally twice a day. Common side effects such as abdominal cramps, anorexia, diarrhea, nausea and vomiting are also reported. For this drug there are several studies and clinical trials have been done but still there is no high quality data

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published or reported about the efficacy of this drug in terms of treating CoV-2 effectively.[27] [28] [29] [30] [31] [32] [33] [34] [35]

Ribavirin

It is an analogue to guanine. According to studies, it's highly effective against CoV-2 as it inhibits the RNA dependent RNA polymerase. But it is required to be dosed in high concentrations for invitro activity against CoV-2 to inhibit viral replication. The dosage is nearly 1.2 to 2.4 grams orally in every 8 hours with combination of therapy. Some studies suggests it can also be given intravenously or external administrations but in combination with interferons. This drug is having a limited value of treatment because it causes sever dosage dependent hematological toxicity resulting in hemolytic anemia. [36] [37] [38] [39] [40]

Antiretrovirals (Lopinavir/Ritonavir/Darunavir)

These drugs are approved by US Food and Drug Administration (FDA) approved oral combination for the treatment of HIV. There is no published data found for effectiveness towards coronavirus but in some articles it had demonstrated invitro activity against some other CoVs via inhibiting 3-chymootrypsin like protease. In some clinical reports it is found to be effective on few patients who received the drug therapy within 12-14 days. The recommended dosing for this drug is 400mg/100mg twice daily up to 12-14 days. Similarly other antiretroviral drugs can also be effective via enzyme inhibition mechanism but there is no published data found. [41] [42] [43] [44] [45]

Umifenovir (Arbidol)

According to many different studies this drug is one of the promising repurposed antiviral agent with a unique mechanism of action against the virus particles. It targets the S-protein/ ACE2 interaction and inhibiting the membrane fusion of viral envelope. The suggestive dose for this drug is 200mg orally in 8 hours. There is a limited clinical data available of using this drug as a potent antiviral agent in terms of treatment of CoV-2. [46] [47] [48] [55]

Favipiravir

It was previously known as T-705. It is known as prodrug of a purine nucleotide (Favipiravir ribofuranosyl-5'-triphosphate). This antiviral agent inhibits the RNA polymerase of the virus and halts the whole viral replication mechanism. Previous studies have shown its effectiveness in treatment of viral diseases such as Influenza and Ebola.

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The loading dose is of 2400mg to 3000mg every 12 hours in 2 times followed by maintenance dosage of 1200mg to 1800mg in every 12 hours. There is a limited clinical data has been reported for the use of this drug in the treatment of CoV-2. [49] [50] [51] [52] [53] [54] [55]

Remdesivir

It is known as GS-573U (monophosphate prodrug). It is highly effective towards RNA viruses (Flaviviruses, Coronaviruses). This drug was very effective during the Ebola outbreak. Due to its broad spectrum potential it is a promising drug in treatment of CoV-2. Many studies have been shown that this drug prevents lung hemorrhage and reduce viral titers in lungs comparatively to other drugs. This drug acts as an analogue for Adenosine Tri Phosphate (ATP) and competes naturally with ATP substrate for incorporation into nascent RNA chain of CoV-2 RNA dependent RNA polymerase, resulting in the chain termination, halting the viral replication process. The loading dose of this drug is 200mg to 100mg IV infused over 30-120 minutes with or without require mechanical ventilation for 1-10 days. [56] [57] [58] [59]

Blood Thinners

A research reported in Journal of the American College of Cardiology some patients were tested and treated with blood clot preventers which decreased the death rate by 29%. But the study was not randomized so it cannot prove that blood thinners are directly effective on CoV-2.

Plasma Therapy

According to some studies done in China severely ill CoVid-19 patients were treated with convalescent plasma (CP). The patients had shown significant improvement with in 3 days with raised antibodies titer and reduced viral load. This therapy involved taking plasma from the cured CoVid-19 patients and injecting it directly to the blood stream of the suffering patient. It is reportedly highly effective therapy for the treatment of CoV-2.

Other Therapies

Researchers trying to find different better ways to treat this sever disease but in absence of proven therapy there are some adjunctive therapies used namely Corticosteroids therapy, anticytokine or immunomodulatory agents, and immunoglobulin therapy.

Nanotechnology a future prospect:

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Nanotechnology can be used to develop nano-vaccines which can be very helpful in the treatment of CoVid-19. According to a recent study an idea is proposed to design a nano-vaccine using viral proteins through which immunogenic peptides can be presented both on surface or encapsulated form. It will consist of multiple epitopes which can be both presented as adsorbed or encapsulated and can be studied both invitro and in vivo to check the immune responses raised by the viral epitopes. Nano-vaccines may be non-infectious as they cannot revert to virulent state and there is a very less risk of allergy.

Prevention: [2]

The novel corona virus is a great problem of this year and till today there are very less clinical approved treatment available for this infectious disease and the prevention is also very crucial at this stage. As different properties of this virus makes the prevention difficult and less effective as at each and every stage of this disease the infected person is spreading the virus whether its initial stage or its clinical recovery stage. The effective prevention of this infectious disease is only can be done by following the proper sanitization guidelines provided by the World Health Organization.

Isolation of the infected person is mandatory. Suspects with mild symptoms should kept under surveillance of health worker. Proper ventilation should be provided to the infected person. Patients should be asked to cover their face with a protective shield/ cover. Care takers should avoid close contact. Health care workers, care takers of infected person should cover themselves with PPE suit or recommended facial mask and shield. Proper sanitization should be done nearby patients. After coming in contact with any of suspect or surface area hand should be washed and sanitized properly before touching mouth, nose or eyes.

Family members and relatives of the suspect and infected person should go through suggested tests and available preventive measures. Avoid large gatherings, unnecessary travelling to infected/ contaminated area or city. Hygiene practices should be followed properly for coughing, sneezing and sanitization of areas.

All of health workers, hospital staff, other officials should keep themselves updated about any kind of pandemic disease to prevent spillover.

Conclusion:

This review enlighten about the general information about the novel corona virus, how it become pandemic and the available mediums of treatment and preventive measures.

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Covid-19 has challenged the world economic, medical and public health infrastructure. This pandemic had shown that how a virus can turn the tables and bring crisis in the global healthcare sector. There are several ongoing researches to find a treatment but in past year there was no effective clinically assured and approved treatment available which lead to globle crisis. The vaccines and specific drugs are avilable in the market which is approved to provide required protection. But such viruses are threat to life because of there capability to mutate. So, it is recommended to be aware about infectious disease, their symptoms, treatment and preventive measures to avoid such pandemics in future.

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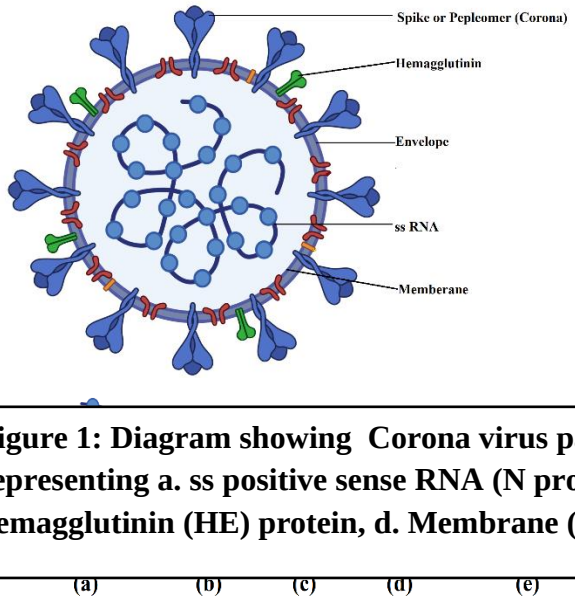

Figure 1: Diagram showing Corona virus particle having enveloped spherical structure representing a. ss positive sense RNA (N protein), b. envelope (E) protein, c. hemagglutinin (HE) protein, d. Membrane (M) protein, e. Spike (S) protein.

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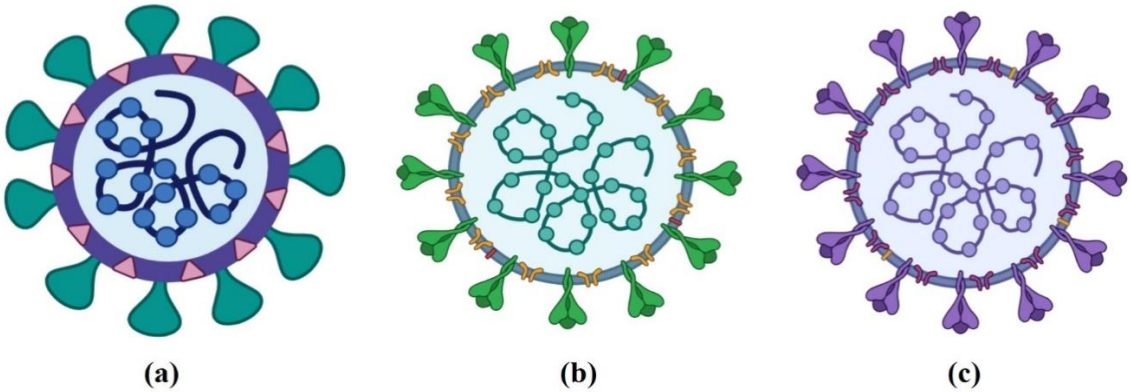

Figure 2: Diagram showing Different structures of Corona Virus Particles.

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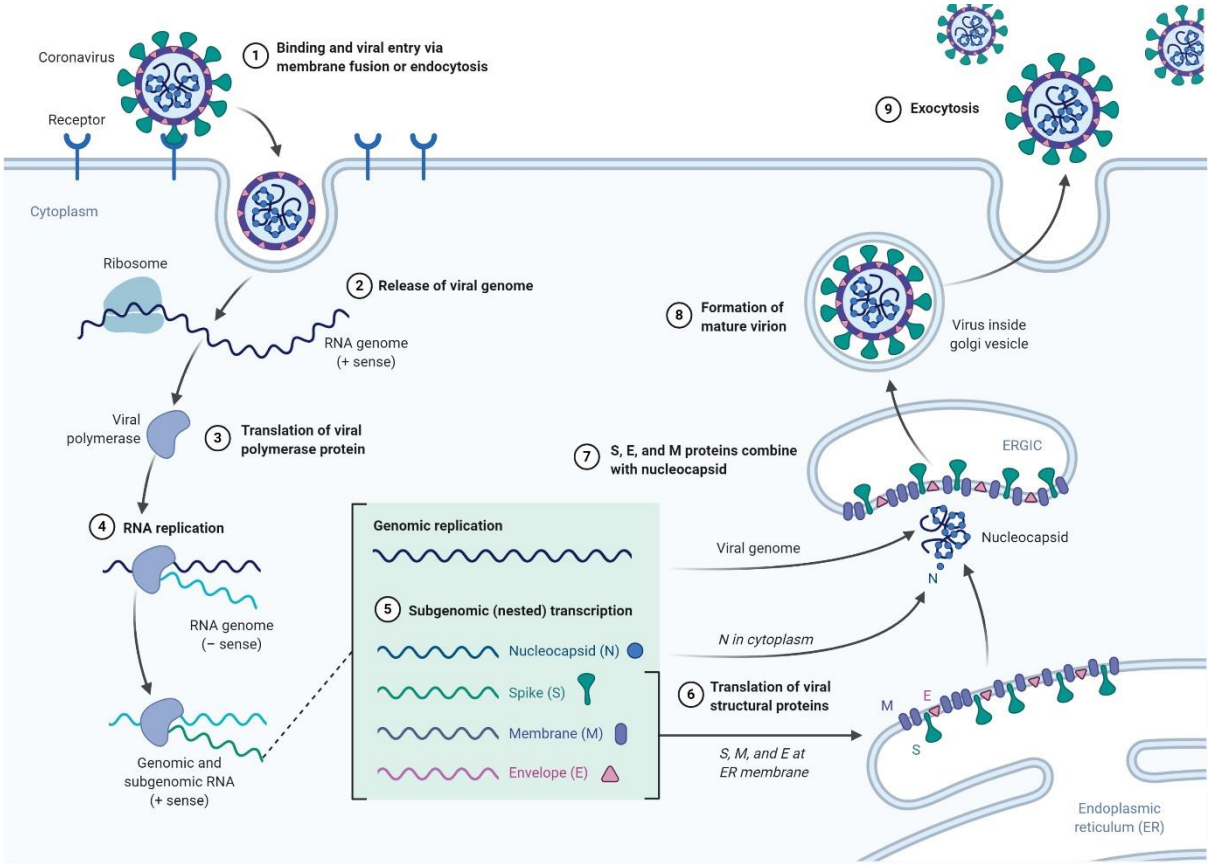

Figure 3: Diagrammatic representation of Replication of COVID-19.(created with biorender.com)

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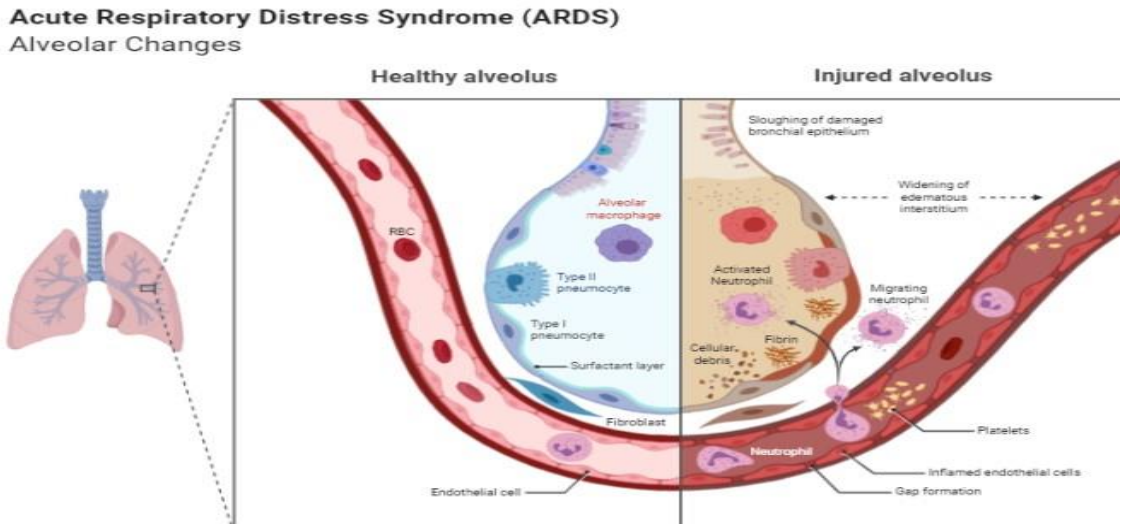

Figure 4: Diagrammatic representation of Acute Respiratory Distress Syndrome. (created with biorender.com)

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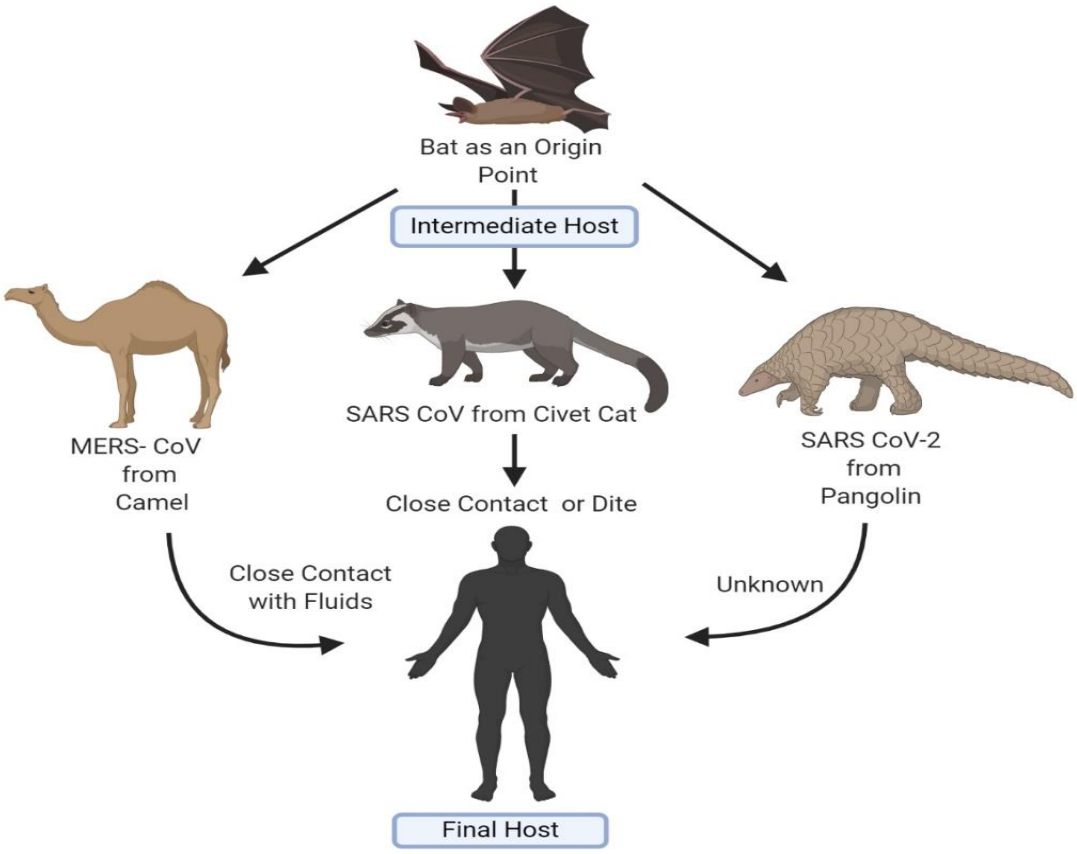

Figure: Diagram showing the possible transmission flow of COVID-19 virus.(created with biorender.com)

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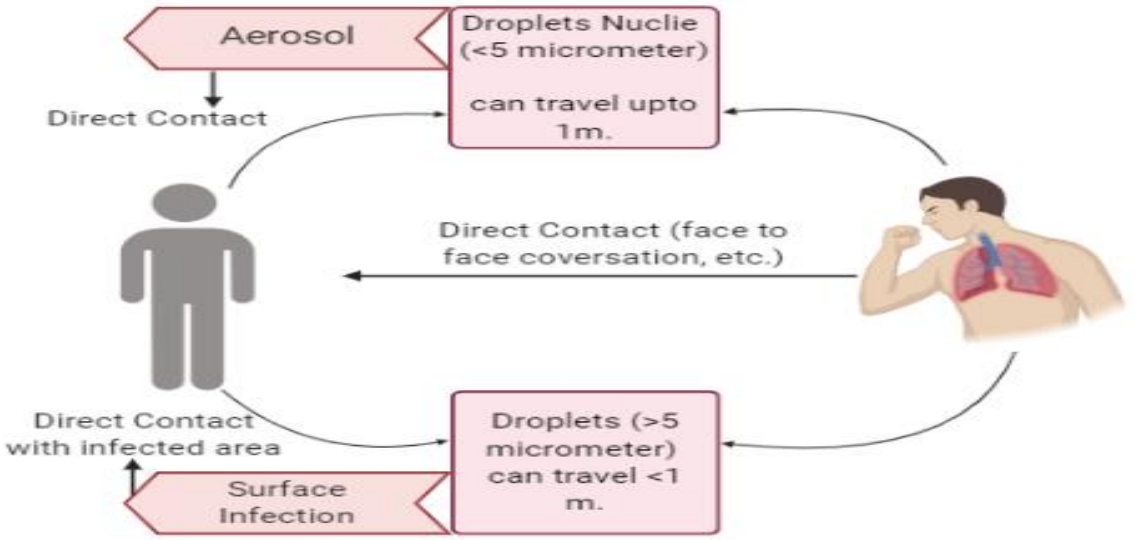

Figure: Diagram showing transmission of COVID-19 through an infected person both directly and indirectly.(created with biorender.com)

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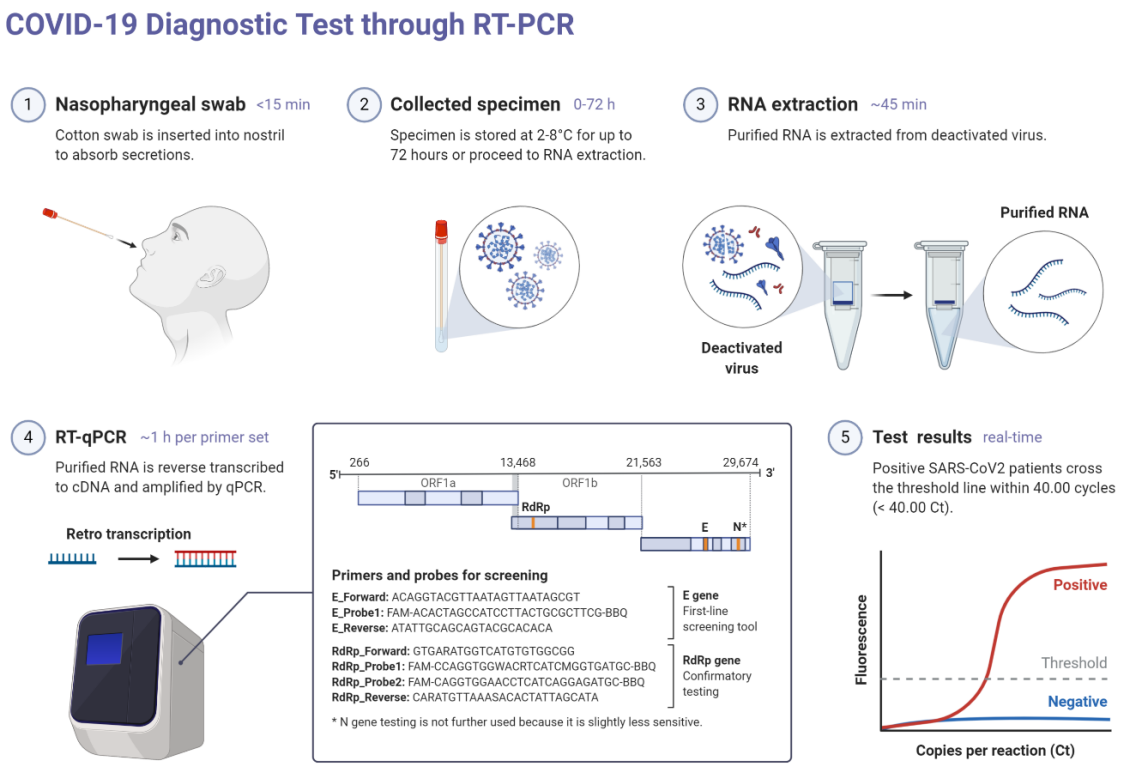

Figure: Diagram showing the diagnostic test for COVID-19 through RT PCR. (created with biorender.com)

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