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Methods of preventions of viral contamination from various surfaces using radiations and mechanic waves: Review

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ABSTRACT

Virus is microparasites capable of multiplication in living cells only. They use cell machinery to reproduce themselves. Most viruses consists of genetic material as DNA or RNA that can be either double or single stranded which is covered by protein or lipid capsid. Complexity of encoding enzymes can range from 4-200 proteins. Viral attack can induce innate immunity and mount cellular and humoral immune response No antibiotic can show lethal action to virus hence physical, mechanical and chemical killing of virus is done. In this paper we will discuss about eradication of viral particles using mechanic waves and radiations such as microwave, electromagnetic radiations, laser pulse, ultra violet radiations, ultra sound waves etc.

Keywords: Virus, Decontamination, radiation, waves, UVC.

INTRODUCTION

In past few years, many efforts have been taken to resist airborne diseases caused by virus such as SARS (severe acute respiratory syndrome) corona virus and influenza virus. These viruses could cause catastophic illness in worldwide and harm population at large scale. These viral epidemic preventions are required in open public area with high efficiency devices. The prevention steps include strong chemical inactivation, microwave heat treatment and UV radiations, however all these methods affects public area and population as well. Viruses are capable of absorbing ultrasonic waves and can be inactivated by generating corresponding resonance ultrasound vibrations in GHz region [2]. In 2015, Szu-chi- Yang suggested that structure energy resonance transfer (SRET) can be effective to inactivate virus by using microwave thermal heating at particular frequency. Theoretically this SRET process is an efficient way to excite the vibrational mode of whole virus structure due to 100% energy conversion of a photon to phonon of same frequency. The SRET, however have some limitations and got affected due to surrounding environment which influence the quality of oscillator

(Virus). It is important to study the behaviour, phenomena, threshold and impact of SRET as they are being exposed to public area. The study of relation between induced stress and field magnitude of the illuminating microwave is necessary since the virus could be inactivated when the induced stress fracture the conformation of viral particle.

Ultrasound is another way to disintegration of virus structure which are having highly symmetric structures (icosahedral) such as Herpes simplex virus [4], because of buckminsterfullerene type fullerene which has soccer ball or cage like structure which show high symmetry for disintegration of icosahedral viral molecule. A fullerene molecule consists of 60 atoms of carbon having high frequency of disintegration of molecule. A similar simplicity can also be expected in HIV viral molecule [5]. The frequency of disruption can be calculated by using sound wave equation $f=C/\lambda$, where f is resonant frequency velocity, C is velocity and λ is wavelength [6]. By analogy it is said that the virus resonant ultra sound energy would be absorbed preferentially by virus which may lead to their inactivation and partial damage.

In another study Constantinos V. Chrysikopoulos use the high frequency ultrasound in combination with visible light. Their studies were done in order to detect and decontaminate the water borne virus. However commonly used disinfection processes were using since long ago that includes ozonization, chlorination, and ultraviolet radiation already but it is important to note that these chemical methods of disinfection is harmful to the users as well. Apart from this, several more expensive methods have been introduced such as streamer corona discharge, high energy electron beams, photocatalysis, irradiation, ultrasound, gamma radiation and many more.

One more experimental step towards this goal of prevention is to use microwave radiation absorption. Microwave helps to transfer microwave excitation energy to vibrational energy of microorganisms. Impulsive Stimulated Raman Scattering (ISRS) allow a viable way of producing large amplitude vibrational mode in solid state system as well as in liquid state [7]. KT Tsen in 2007 demonstrated that M13 bacteriophage at a particular pfu concentration is helpful in controlling and inactivating the unwanted microorganism. The study shows that the use of visible femtosecond laser system to excite a coherent acaustic Raman active vibrational mode in M13 phages through ISRS to such a high energy state to inactivate virus. In addition, since structural change due to the mutation of microorganisms leads to slight

differentiation of the vibrational frequency of their capsids, damage caused to viruses through vibration of their mechanical structures likely would not be immune to simple mutation of receptors on their cell surface and similar treatment procedure remains active.

A most striking way to kill virus is the exposure of sunlight or can say solar UV radiation which acts as principal and abundant natural virucide in environment. UV radiation inactivate the virus by changing their structure of DNA or RNA. The most effective wavelength at which virus can be inactivated is at 265 nm [8] that fall in UVC range whereas UVB and UVA portions of the spectrum, 290 to 320 nm and 320 to 380 nm, respectively [9]. However UVB and UVA also show effect on viral DNA but with lower efficiency. C. David Lytle, 2005 studied that on exposure to UV_{254} radiation on a low pressure mercury vapour (Germicidal lamp) with the primary exposure at 254 nm. However UV_{254} is not found in the sunlight which reaches to the earth's surface, the ground level virucidal solar UV wavelength fall above 290 nm [10].

Fortunately the primary photochemical process can damage the DNA or RNA The nucleic acid in virus particle plays an important role in the absorption of UV radiation and in its inactivation. In most viruses the other major constituents of the virus particles play relatively minor roles in inactivation by UV [8]. The number of bases in DNA or RNA is important for determination of sensitivity to UV inactivation, because the more target molecules, the more likely the genome will be damaged at a given wavelength of UV exposure. Another noticeable difference in sensitivity between viral nucleic acid types occurs because the most common lethal photoproducts of UV are pyrimidine dimers, particularly thymine dimers [11]. The DNA containing virus show more susceptibility than RNA containing virus because of presence of thymine [12, 8].

These sensitivities can be used to predict the sensitivities to UV 254 of viruses of particular interest in biodefense, including Ebola, smallpox, Marburg, Junin, Congo Crimean, and other Venezuelan equine encephalitis and hemorrhagic viruses. Vaccinia virus also show significant inactivation at Upper-room 254-nm germicidal UV (UVC) light and economical means of air disinfection for tuberculosis and other airborne infections [13, 14, 15,16]. If organisms circulate from the lower room to the upper room (i.e., if there is adequate mixing of room (atmospheric) air) and receive an adequate dose of UVC, upper-room UVC can potentially lower the concentration of infective organisms in the

lower part of the room and thereby control the spread of airborne infections among room occupants without exposing the occupants to a significant amount of UV radiation [13, 15, 17].

Do-Kyun Kim, 2018 also reported that Severe Accute Respiratory Syndrome- CoV (CoronaVirus) IN 2003 and Swine flu influenza virus H1N1 in 2009 stimulate the process of disinfection and development of air purification system to control microorganism such as bacteria, virus and fungi [18]. Dimerization of pyrimidine disturbs DNA replication and transcription, which leads to cell death [19, 20, 21]. Until now,UV irradiation has mostly been performed with conventional low-pressure mercury UV lamps (LP lamps), which emit a 254-nm peak wavelength.

Conclusion: It has shown that micoorganism such as bacteria, virus and fungi have particular range of inactivation in case of radiations such as microwave, electromagnetic radiations, laser pulse, ultra violet radiations, ultra sound waves etc. Few works demonstrated the modification in the study to eradication of viral contamination in open air, in solid state or in liquid state, in aerosols (tiny droplets released during sneezing of infected person), although the viral contamination can only be destroyed at the ground level but cannot alter the immune system. The excitational or vibrational energy disturb the conformation of viral capsid, whereas other radiations such as UVC cause the structural changes in their genetic material like DNA and RNA. Use of open air source of radiations or closed chamber can be effectively fitted in public area to control and inactivate viral particles.

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Type-2 Diabetes: Abnormalities associated with the elevation in level of liver function tests (LFTs)

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ABSTRACT

Liver Function Test (LFTs) is very common for the primary analysis of any types of malfunction associated with liver. For screening of liver in clinical practice, widely used LFT includes- ALT (*alanine amino transferase*), AST (*aspartate amino transferase)*, Alkaline Phosphate (AP), Bilirubin, Albumin and Prothrombin Time (PT). In type-2 diabetic patients, a nonspecific Gamma-Glutamyl Transferase (GGT) marker is increased. While analyzing the epidemiological studies, intake of alcohol, cigarette smoking, BMI, Systological Blood Pressure, Coronary Heart disease, Heart Rate, hematocrit, uric acid it having the positive association. But at the same time it shows the inverse effect with the physical activities. GGT is proposed as another marker for insulin resistance because the GGT increases in diabetes. It is concluded that the individuals who have type-2 diabetes seems to have higher incidence of LFT abnormalities than the person who are not diabetic. For more than 6 months, elevation of ALT is commonly observed. It develops a mild chronic elevation & it should be screened for treatable cause of chronic liver disease like in hepatitis-B and C. Patients having regular monitoring generally donot observe any elevation in level of LFTs. Before starting any oral anti-diabetic or lipid modifying therapy, a proper clinical judgment should be required. Elevation of *transaminase* not always correlated with histological changes in liver. But at the same time a fall in level of ALT is achieved in blood while giving anti-diabetic agent to patients.

Keywords: LFT, Antidiaibetic, Drug therapy, lipid modifying therapy.

INTRODUCTION

Liver function test (LFTs or LFs) also called as hepatic panel; it gives us the information at clinical level by proper routine diagnosis of blood serum of patient's liver. A proper monitoring should required for the earlier detection of any malfunction associated with the liver. Type-2 diabetes Mellitus (T2DM) is a chronic, progressive and serious metabolic disorder

characterised by hyperglycaemic disorder (high blood glucose levels) & associated with numerous complication & co-morbidities, including cardiovascular disease, nephropathy (kidney damage), neuropathy (nerve damage) & retinopathy (retinal damage). Global prevalence of the disease has risen rapidly in the past several decades, primarily as a result of rising obesity a major risk factor for T2DM. Commonly perform LFTs which are serum aminotransferases, alkaline phosphatase, bilirubin, albumin & prothrombin time. Aminotransferases, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) which act as maker for hepatocyte injuries and it measure the concentration of intracellular hepatic enzymes. It leaked out during blood circulation for billiard functions and cholestasis. Alkaline phosphatase (AP), μ-glutamyl transpep- tidase (GGT), and bilirubin act as marker and for the synthetic function albumin & prothrombin are responsible. The amino transferase AST and ALT are normally range for < 30-40 units/l. An acute viral hepatitis, ischemic hepatitis drugs-or-toxin induced liver injury when the normal upper ranged limit is greater by 8 times. A chronic mild elevation of aminotransferases, or AST and ALT< 250 units/l for >6 months are much more common among the patients with acute hepatitis.

This article will provide us a review on the clinicopathological investigation related to the incidence, causes and drug therapy which associated with the type 2 diabetic patients along with their elevated LFTs level. In type 2 diabetic patients a chronic mild elevation of transaminases are found generally.

LFTs Elevated in type-2 Diabetes

During fasting & postpraudrial state, liver help to maintain the normal blood glucose concentration. A reduction of insulin level in liver it increases the glycogenolysis & hepatic glucose production. Liver externalised the conditions which depict by the insulin resistance & are distinguishable earlier than fasting hyperglycaemic. The triglyceride storage & lypolysis abnormalities found in liver which is an insulin- sensitive tissue. However, it is unequivocal that the methodical genetic events, environmental, metabolic factor & sequence of the events lead to the cardinal insulin resistance [1].

Chronic hyperinsulinemia is found to be more liable towards the liver relative resistance of insulin for the animal models.

This signalised the failure of insulin signal by increasing the insulin receptor substrate-2. The process of lipogenesis is increased by the up gradation of sterol regulatory element-binding protein 1c (SREBP-1c) [2]. Promoting the fatty liver & increase the triglycerides availability by the de novo lipogenesis in the liver responsible for the regulation of SREBP-1c. Hence, down the regulation of insulin receptor substrate-2-mediated insulin signalling pathway in insulin resistance states. As the VLDL assembly & secretion also increased [1].

Automatically it becomes toxic for hepatocytes when the excess of free fatty acids found in the insulin-resistance state. The keys processes involved in the regulation of metabolism the eminent mechanisms which include cell membrane disruption at high concentration, mitochondrial dysfunction, toxin formation, activation and inhibition [3]. Other feasible interpretation for elevated transanimases in insulin-resistance states which involve the oxidant stress from reactive lipid peroxidation, peroxisomal beta-oxidation and recruited inflammatory cells. The insulin- resistance state is delineate by an increased increase in proinflammatory cytokinesis such as tumor necrosis factor-a (TNF-a), and marked the hepatocellular injury. In primary stage studies, it propound a possible genetic link or inclination to fatty liver as the increased frequency of specific TNF-a–promoter polymorphism was found in nonalcoholic steato-hepatitis (NASH) patients [4]. All the above theories allege elevated transamination to direct hepatocyte injury. The impairment in insulin signaling rather than purely hepatocyte injury which is marked by gluconeognesic enzymes whose transcription is suppressed by insulin and it also hypothesized the elevation in ALT level [5].

Elevated LFTs can Prolongate Diabetes?

In type-2 diabetic patients a non-specific marker GGT level is increased. In epidermiological studies, it association is positive with cigarette smoking , alcohol intake, heart rate, BMI, systolic blood pressure, coronary heart disease, serum triglyceride, uric acid & hematocrit. It has a direct involvement with the physical activities [6]. As GGT level rise in diabetes & it results increased in BMI. Hence, it has been introduce as another marker of insulin resistance. A prospective cohort study of 7458 non-diabetic men aged 40-59 years was conducted for 12 years. It helps to evaluate the GGT which predict the development of type-2 diabetes while predicting a model as the GGT was added,

but there is no process of progress reported in BMI strength and glucose for antipating the development of type-2 diabetes.

In non-diabetic Ohlson et al found ALT a risk factor develop for type-2 diabetes swidish men, independent of obesity, body fat distribution, plasma glucose, lipid, AST, bilirubin concentraction & family history of diabetes [8].

Same result found, Vozaroza et al it took 451 non-diabetic Pima Indians for approximate 6.9 years it allocate whether the development of type-2- diabetic linked by the hepatic enzyme elevations [9].

Percent body fat related to baseline of ALT, AST and GGT elevated ALT at baseline associated with the increased in hepatic glucose output. Arrange the whole body weight, age, sex, body fat, sensitivity and acute insulin response as hepatic insulin sensitivity risk of type-2 diabetes decline. It shows the direct involvement. It has an immense potential role to increase the hepatic gluconeogenesis or inflammation in the pathogenesity of the type-2 diabetes. As the author determined that higher ALT level regards the more risk towards the type-2 diabetes.

Diabetes Phenomenon over Elevated LFTs

Salmele et al studied based on the clinical findings of 175 unselected diabetes outpatients in Firland it has direct dissemination of LFTs abnormalities [10]. 118 patients were took up & found to have type -2 diabetes and 57 marked for type-1 diabetes. Out of 118 patients only 33 patients of type-2 diabetes used insulin in addition to diets and oral hypoglycemic drugs including metformin & sulfonylurea. Hemoglobin A_{1c} (A1c) averaged 11.2% ± 2.4%. None of the patients had clinical signification for diabetic nephropathy (diabetic kidney disease) and chronic liver disease. LFTs clinical assessments included the albumin, ALT, ALT, AP, GGT, serum concentration of cholic acid, total bilirubin and chenodeoxycholic acid. 175 diabetes outpatients (100 subject) it regard 57% from the total abnormalities corresponds to LFT, 27% (48 subject) with two abnormalities. The type-2 diabetic patients more frequently had elevated ALT (22.9 Vs 5.3%) & GGT (23.7 Vs 10.5%) level than those with type-1 diabetes.

Type-1 diabetes patients more frequently observe the elevated bilirubin level (21.1 Vs 10.2%). The increased LFTs level is more than the twice of upper limit of normal

range. The most significant variation associated with ALT & GGT. The analysis shows that the BMI >25 kg/m2 and poor diabetic control (fasting blood glucose >216 mg/dl). As ALT elevated if directly linked with the onset of diabetic since last 4 years. Use of proper diet or sulfonylurea is given on the mature onset of diabetes (35-51 years). Examine the massiveness of LFTs estimate all the histological changes, Salmela et al studies looked up for 72 sequential diabetes inpatients with hepatomegaly or abnormal LFTs as patients who were awaiting liver biopsy [10]. The type-2 diabetes patients are 68 and 4 had type-1 diabetes, but all of them had abnormal LFTs and hepatomegalay. All have normal blood counts, serum, electrolytes & renal functions. But no case reported for the heart failure. Only 5 of them had a history of social drinking alcohol and rest 67 as abstainers. 63 patients had abnormal liver histology. 48 had liver fatty liver or steatosis with non-specific inflammatory changes. 14 evidence of fibrosis reported. GGT & ALT it seems too elevated generally but here is no significant difference in mean values of ALT & GGT. The histopathology worsened (steatosis to inflammation to fibrosis). Abnormal LFTs result is common in diabetes & particularly in over weight type-2 diabetic patients. Further there is no creditability for histological changes in the liver. Erbey et al in a large group study it analysed 18,825 non-institutionalized patients with an oversampling of Mexican Americans & African Americans [11]. Total sample study in which 4.1% elevated ALT, 6.7% type-2 diabetic patients out of which 7.8% had the elevated ALT and rest 3.8% prevalence in those free from any type of diabetes. The propagation in the ALT elevation level when greater by 3 times than the normal value & have no significance difference between diabetic & non-diabetic patients (0.4 Vs 0.7%) and for the obese (BMI >30 kg/m2) & for over weight (BMI >25-30 kg/m2). They had more elevation in ALT. 10.6% prevalence in obese diabetic versm a 6.6 prevalance in obese non-diabetic patients.

Type-2 Diabetic: NAFLD

NAFLD (non-alcoholic fatty liver disease), it is the most common reason for the elevation of LFT s level in type-2 diabetic patients. In clinicopathological investigation NAFLD represent a broad spectrum of histological evidence from hepatic streatosis or fat accumulation in hepatocytes without any inflammation, to hepatic steaotsis with a necro-inflammatory component that may or

may not have fibrosis or NASH. Low or absence of alcohol consumption characterized as NAFLD with or without necro-inflammatory activities shows the macrovesicular steatosis & cast off the other forms of liver disease. Indecipherable the pathogenesis & it marked by the deposition of triglycerides within the hepatocytes. For the triglycerides deposition insulin resistance play a major role. As inflammation followed by the ATP depolarization, mitochondrial dysfunction, FA, excess intercellular & oxidant stress [3]. NAFLD is regared as the most common in patients with having elevation of serum aminotransferase ranges from mild to moderate. There is no direct intensification accordance to the histology of liver as transaminase elevation in NAFLD [12].

Non- Diabetic: NAFLD

Diabetic and non-diabetic chronic elevated LFTs in the United States is one the most effective etiology as NAFLD is replacing alcohol & viral hepatitis [3]. Among all the patients reported NAFLD, 60-95% are obese, 28-55% type-2 diabetes & 20-92% have hyperlipidemia. Further study conducted were 1,124 adults examine and they shows the evaluation of chronic elevated LFTs. Based on absence of serum markers for infection (hepatitis B & C), 81 were rectify with undetermined etiology or hereditary cause of liver disease (α-1-antitrypsin, iron, ferritin, iron binding capacity, ceruloplasmin etc) metabolic (TSH- thyroid stimulating hormone), autoimmune (anti-smooth muscle antibody, anti-mitochondrial antibody, electrophoresis, serum protein) [13]. No history reported by the chronic liver disease & non for alcohol or hepatotoxic drugs. No sarcoid in chest X-rays for all the patients. There is no evidence in article for the transaminites like celiac disease, renal insufficiency and muscle disorder. With no identified etiology of elevated liver enzymes 81 patients marked negative, abnormal history in 73 patients, all had some association to steatosis. The patient has some association to steatosis. The prevalence rate of stratosis is 50.6% & steatohepatitis is 32% but without any clarified etiology for liver disease.

With diabetes and without diabetes for individuals, same study conducted for 354 patients, to investigate abnormal LFTs over liver biopsy underwent. Steatosis and steatphepatosis on biopsy evidence excluded 66% of the patients for specific diagnosis, since their serological & clinical reports available [14].

Type-2 Diabetic and HCV Projection

In united state, predictor of type-2 diabetes is known to be independent and the most alarming cause for liver disease is hepatitis C- virus (HCV), without cirrhosis it is the most common endocrine disease within diabetic patients with high prevalence for HCV reported [15], [16]. Risk factor of acquiring HCV when comparing 176 diabetic patients to 6172 blood donors matched & it shows higher prevalence of HCV infection, diabetic patients (11.5 Vs 2.5% $p<0.001$) [17]. 72.3% had abnormal elevated LFTs, with HCV diabetic patients on comparing it with diabetic patients with no report of HCV ($p< 0.001$) it shows 27.7% impact. The study gives us the idea about screening is important for HCV among all the diabetic patients with elevated LFTs.

Elevated Transaminases Type-2 Diabetes with Statin

It not show any significant association with the heart protection study of 20,536 who has the higher risk individuals of vascular disease among all diabetes patients. The elevated rates for ALT are 2 times the upper limit of normal range were 1.8% in simvastatin group & 16% in the placebo group in the pravastation in Elderly Individuals at risk of vascular disease (PROSPER) trial, ALT or AST level is more than 3 times the upper limit of normal range of one patients in placebro group & in pravastatin only one patients reported rhabdomyoloysis [18], [19]. In pravastatin 36 patients had myalgias, compared it with placebo group only found 32 patients. Association of high dose statin therapy with more frequent abnormalities of LFTs patients with clinical cardiovascular disease (CVD) were randomized to 10-80 mg of atovastatin while treating to new targets (TNTs) trail thrice the upper limit of the normal range obtained for the incidence of persistent elevation in AST & ALT or both observe for 4-10 days and range obtained is 1.2 and 0.2% respectively ($p< 0.0001$) [20]. Recommendation based on the current large trails from the American College of physicians for type-2 diabetic patients with cardiovascular risk factors also in order to avoid any other severe disease. In major issues such as macro-vascular complication statin used as primary prevention. Routine monitoring of LFT not required in these patients, even the statins and other drugs should be avoided until the baseline abnormalities found in LFTs & myopathy as it can increase the other adverse situation too [21]. It should be advised not to use the advance statin therapy as long as patients are

monitored carefully, as for diabetic patients the baseline transaminase less than three times the upper normal limit. But there is disagreement over monitoring recurrence of these patients. Other disagreement builds on elevation of statin hepatotoxicity whether it developed by transaminase or not? [22]. The proven benefits from CVD risk reduction is less weighted over the known potential risk of statin therapy by the major possibilities of hepatotoxicity, among the diabetic patients over age of 40 years who have a multiple cardiovascular risk factors called as CVD.

Elevated Transaminase When Type-2 Diabetic Patients Administrated With Oral Agents

The sequential report of hepatotoxity led Jick et al which is introduced by the insulin sensitizer in type-2 diabetic patients to analyzed the baseline risk of liver disease on oral agents other than thiazolidinediones [23]. General Practice Research database UK based researcher identified 40,190 type-2 diabetic individuals treated with oral diabetic agents, which include metformin, guar gum & sulfonylurasa in between years 1989-1996. When the oral therapy began none of the patients reported the known liver disease. During the study periods out of 605 cases only 1.5% individuals identified as new diagnosis of liver disorder, 249 (41.2%) attributed to a predisposing conditions, 186 (31%) as mild asymptotic liver enzyme abnormalities with no clinical relevant, 113 (18.7%) had a specific non drug etiology listed. The rest 57 (8.7%) are no predisposed conditions with a clinical relevant of liver disease which attribute towards the other drugs, fatty liver & unknown. An incidence of 0.002/100 person years these two cases oral antibiotic agents not to be ruled out.

Rajagopalan et al comparison in between pioglitazone v/s oral antidiabetic agents claimed date based on the incidence of liver failure in type-2 diabetic patients [24]. As report received by the pharmacy on their first antidiabetic treatment it divided into different group based on antidiabetic therapy. If the patients belong to group pioglitazone then by the help of metformin & sulfonylurea group they matched with the patients of rosiglitazone. Same characters i.e. clinical & demographic, found within the matched groups, the analysis of patients includes 4.458 similar pairs of pioglitazone v/s rosiglitazone treated patients. 1,474 pairs of pioglitazone v/s sulfonylurea treated patients & 1,137 pairs of pioglitazone v/s metformin treated patients. No patients reported increased risk of liver failure or hepatitis with duration of 2 years by

pioglitazone when it compared with the patients on other antidiabetic agents. Reversal effect of enzymes elevation with all patients was found to be elevated ALT on pioglitazone [25].

Pioglitazone is able to control the double-blind clinical effects in placebo- controls, virtual identical between patients on pioglitazone and those of placebo (0.26 v/s 0.25%), the incidence of elevated ALT values greater than 3 times the upper limit of normal. More than 6000 patients took and studied by Lebovitz et al for the individuals affected with type-2 diabetes either insulin, metformin or glyburide and rosiglitazone placebo used various dosed in double blind clinical trials. 8.5-9% in all groups since from beginning of study the mean Alc level is same [26]. For the first months at every 4 weeks of treatment & then afterwards at a interval of 6 to 12 weeks occurred for the screening, baseline and proper measurement of liver enzymes. If any individuals found to have grater ALT, ALP or AST level by two and half times the upper limit of normal during screening then the individuals excluded from the study. This added with present recommendation when advise not to use rosiglitazone orpiglitazone. Approximate 3800 for at least 6 months, 2800 for one year & 1000 for at least 2 years monitored of all those on rosiglitazone. Among 5,006 patients who went with rosiglitazone none of them had hepatotoxic effects. ALT is thrice the upper limit of normal ranges & rosiglitazone is 0.32 %, placebo group 0.17% and 0.40 % either with insulin or sulfonyureamet formin groups. No difference found for the treatment of placebo, rosiglitazone and other antihpyerglycemic agents as study conducted a respective incidence rates of 0.29, 0.59 & 0.64/ 100 person- years had been reported. As study further proceded 5.6% of individuals whose serum ALT values in between 1- 1.5 the upper limit of normal baselone, the individuals 66% normalized ALT treated with antihyperglycemic medicines & that of only 38.7% normalized ALT they were treated by placebo normalized [26].

Over & over again found mild chronic elevation of tranaminities in diabetic patients reduced by improvised insulin resistance & it has specific support among insulin resistance, hepatic function & glycemic control.

A surrogate, thiadolidinediones for the insulin resistance use to treat NASH it was shown in pilot studies that the decrease in LFTs illustrated with rosiglitazone & pioglitazone therapy for diabetic patients.

For 48 weeks a study took placed 18 non-diabetic patients with NASH on pioglitazone with a daily dose of 30 mg/48 weeks [27]. 72% with normalized and rest all patients with decrease serum ALT level by the end of study it was observed. A great fall found in report of serum ALT level from an average of 99 units/ l to a baseline of 40 unit/l within a interval of 48 weeks.

One more study used rosigitazone for 48 weeks treatment on 30 patients with NASH a daily dose of 4 mg & impairment of glucose tolerance or diabetes among 50% of them [28]. A significant improvement were noticed in the level of mean serum ALT levels among 25 patients who finished the study of period 48 weeks with a changed notice from baseline 104 units/l to 42 units/l. Again increased in level of liver enzyme near to pretreatment for rosiglitazone found after 24 weeks offs & this observation made over the end of 72 weeks.

CONCLUSION

The elevated ALT is most common abnormalities among diabetic patients. It concluded that the individual those who having the type-2 diabetes follow the higher incidence of LFT abnormalities than the person who do not have diabetes. After proper clinical observation for more than six months if any mild chronic elevation of ALT or elevation of ALT≤ 250 unit/l in any diabetic patients found then the screening for treatable cause of chronic liver disease like hepatitis B, hepatitis C and hemochromatosis seems to have incidence in type 2 diabetes. The diagnostic workup is probably not required for those patients who do not have any evidence for more serious liver disease, such as elevation in bilirubin or prothrombin time or decrease in albumin or even in a patients who have any direct medical history & physical examination do not raise suspicion of other cause of elevated LFTs. If patients develop any preliminary symptoms which enhance the hepatic impairment before administering drug therapy, a proper routine monitoring of LFTs in patients with type 2 diabetes it requires. Time to time screening based on the clinical assessments. So, nod your head that transaminases does not always have correlate with the histological changes in the liver. As higher glucose level achieved then descends in level of ALT shown by antidiabetic agents. If elevation of ALT is more than three times the upper limit of normal range then it would not be an antipathy to start any oral antidiabetic or lipid modifying therapy.

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IMPACT OF COVID-19 ON OUR ENVIRONMENT

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ABSTRACT

Within a span of sixty-ninety days, the world has been changed totally. Millions people expired already, and several lakhs are infected, and the numbers are growing continuously worldwide. Reason behind all these, is COVID-19 pandemic and spread of Corona virus. Along with human, our environment has also changed a lot during these months. The rate of pollution of air, water, soil, noise has reduced to a great extent. Sustainable development has been autogenerated in the environment and Mother Nature has cured herself a lot. Clear water, view of snow-capped mountains, visibility of wildlife in locality, appearance of endangered animals, pure oxygen in the air all have returned somehow during the lockdown phase throughout the world. On the other hand, increase in biomedical waste, plastic wastes have been a major concern against the environment. These are not only polluting the nature but also creating havoc damage to the aquatic flora and fauna. Present paper has tried to put some light on the effect of COVID-19 on our environment.

Keywords: COVID-19, Pandemic, Pollution, Waste, Corona, Environment

INTRODUCTION

Effect of COVID-19 pandemic on our environment is highly significant. As per the experimental analysis and evidences, release of various greenhouse gases like CO_2, CH_4, CFCs have come down to a significant quantity worldwide during the lockdown phase [1]. Activities in more than 200 countries around the world have become standstill due to the outbreak of Corona Virus in last few months which resulted in some good outcome for Mother Nature.

Impact on the environment:

Factories, institutes, automobiles, refineries, recyclers, agricultural machineries, power plants, mining activities were stopped during lockdown phase against COVID-19 outbreak [2]. Number of vehicles plying on the road came to almost zero in number. All these resulted in the extraordinary downfall of the atmospheric concentrations of CO_2, CO, NO_2, SO_X, Particulate Matters throughout the most populous and most polluted towns of the world. The nationwide

lockdown policy and implementation of law and order to maintain social distancing have resulted in developing waste free roads especially beach areas. As there were no vehicles, aircrafts, trains, buses, commercial and public vehicles on the road, noise level has also been reduced to a great extent especially in COVID-19 infected nations [3].

Cities in China, Italy, England, France, USA, Spain were completely locked down after the first wave of COVID-19 spread out and appearance of Corona virus pandemic. In India, lakhs of migrant workers are approaching several modes of communication to reach home in their native places [4]. Countrywide lockdown has resulted in extreme conditions among countrymen. Worldwide, International and Domestic flights have been closed, trains have been cancelled, inter-state bus services have been stopped.

Discussion

People have been maintaining social distancing, wearing masks, gloves and other personal protective equipment [5]. Staying at home advisory has been strictly implemented nationwide. These were all done to control the spread of COVID-19 in terms of Corona virus. Suddenly, industries, factories, communication and several other and businesses have been shut down which resulted in sharp fall in carbon emission. The lockdown phase resulted in downfall of pollution level into almost 50% than the previous year concentration at the same time [6]. During lockdown phase, vehicular movement was restricted, and industries were shut down. This resulted in the downfall of air contamination to a great extent. Due to suspension of waterways during lockdown phase, neither fishing nor pleasure trips, the water has become clean and in some places crystal clear. In case for Ganga river in India near Haridwar in Uttarakhand water became clean and clear. Animals have been spotted roaming around freely and visiting areas they were not in normal cases. Endangered wildlife was viewed in many places worldwide. Millions of turtles visited land for hatching eggs. Dolphins were viewed in water where they were not seen for decades [7].

COVID-19 and outbreak of corona virus has developed negative effects on our environment. All the affected nations have stopped the waste recycling activities due to safety and security of the workers and employees during the outbreak [8]. Hence, sustainable waste management has not been done at all. Online shopping, packaged drinking water bottles, plastic wrapped food items, groceries, online foods all coupled together to enhance the wastes in and around

us during this lockdown phase. Due to COVID-19, biomedical wastes have increased into several folds [9]. Doctors, nurses, health staffs, patients, COVID suspects, health department officials, police, workers in health centres everybody has generated umpteen number of plastic wastes in the form of gloves, PPE, face shields, masks, shoe cover, head cover, eyeglass etc. [10].

CONCLUSION

It can be concluded by saying that there has been some positive impact observed during the lockdown phase worldwide. These positive impacts of COVID-19 were definitely in terms of nature and environment. Be it decrease in air pollution, reduction in greenhouse gas emission or lowering concentration of particulate matters in the atmosphere. Most populous and most polluted cities in the world situated in India, Italy, France, Spain, England, China, USA, Russia, Germany have observed decrease in the contamination level of air pollutants. On the other hand, quality of water has improved into several fold during the lockdown phase. Several rivers passing through Industrial belts have become clear and clean after the pandemic COVID-19 appeared worldwide. This paper tried to describe the effects of COVID-19 on our environment. Noise level has been decreased and betterment of coastal beach areas were observed as positive impacts during lockdown phase. On the other hand, among the negative indirect effects, the increase in domestic and medical waste was mentioned. It is important to mention that although the emissions of some greenhouse gases (GHGs) have lowered down as a result of the pandemic, this reduction could have little impact on the total concentrations of GHGs that have accumulated in the atmosphere for years. There is requirement of long-term constructive planning on the basis of sustainable socio economic development. There will be disaster if biomedical wastes like used gloves, masks, shoe cover, heal cover, face shields, aprons, PPE kits are not properly disposed and get mixed with other waste products.

At the end, it can be mentioned that that COVID-19 will generate some positive and some negative effects on our environment. Lowering concentration of greenhouse gases for a short time period is not going to be a solution. Corona virus will stay for some more time, and during the unlocking phase of restrictions situation might get worse again in terms of increase in toxicity level of air, water and land. Proper management and long-term planning is the need of the hour.

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Significance of Rhizosphere in Tea Gardens

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ABSTRACT

The lush green undulated terrain of Darjeeling covered with tea gardens is famous throughout the globe for its excellent aroma and taste. The best quality tea is produced in Kurseong hill area under Darjeeling. Experimental analysis on monthly basis was done regarding microbial population in the selected tea gardens. Interactions among certain microorganisms were analysed during the study. Coordination in between specific microbes might also be responsible for the impact. Fungi, bacteria and actinomycetes – these three groups of microbes were tested during the process. Results showed that neo tea plant rhizosphere and rhizosphere of several other perpetual plants, of various ages, flourishing in age old tea gardens, seemed to expediate growth of microbes. At present, tea rhizosphere has been tested thoroughly, specifically in relation to plant-microbe response. Counter to the common outcomes, rhizosphere and soil comparisons were happened to be continuously below 1. The edaphic samples were gathered from vintage tea estates which showed negative impact of rhizosphere. Finding of 'negative rhizosphere effect' in old tea bushes is a significant and a novel nature of tea rhizosphere. Supremacy of a certain population of microbes, affinity towards a section of general opponents, constitutes a good instance of reciprocated selection in natural environment. These discoveries have unlocked newer paths for extended researches in the field of 'rhizosphere microbiology'. Present study is an attempt to evaluate the transforming features and significant implications in the tea industry.

Keywords: rhizosphere, tea, garden, estate, soil, microorganism, population

INTRODUCTION

The Darjeeling logo is a hallmark of excellence. Launched in 1986, the Darjeeling logo has come to represent high quality muscatel flavored tea with the unmistakable class that only Darjeeling can offer. The logo is a significant landmark in the history of the tea industry. Conceptualized by the Association of

Darjeeling Tea Gardens and launched in the international and domestic market by the Tea Board, it guarantees genuine Darjeeling Tea, tested and packed for the connoisseurs of tea throughout the world [3]. The Darjeeling tea industry is a happening place and has more surprises to furnish. The Darjeeling Tea Research Institute is located on Pankhabari Road of Kurseong. Kurseong still applies the traditional basic model of tea manufacture, known as the "Curling, Tearing and Crushing", popularly known as C.T.C [1]. Presently, organic tea is the most famous name given to tea grown using chemical free manure and eco-friendly practices. There is no use of chemical fertilizers and pesticides in an organic tea garden [2].

MATERIALS & METHODS

Regular assessment of various microbial growth in soils growing tea in terms of units making of colony have been executed by the process of plate-count. Three separate soil depths were analysed for the span of 1 year. The examination was done on three groups of bacteria, fungi and actinomycetes [4]. The samples were collected from tea gardens which clearly indicates an overall negative effect of rhizosphere. This negative effect can also be caused by specific microorganism living together [5]. It can be assumed that the rhizosphere of relatively new tea bushes and of various perennial plants having variety in ages generally developed in tea estates which are already established, could have accelerated microbial growth [6]. The -ve impact of rhizosphere of relatively much aged tea gardens do not seem to be a regular incident like growing old in normal condition but could be one of its kind and specific to tea plants [7].

Several experiments were conducted by gathering edaphic samples from various sites of the country [8]. The results showed various distinguishing characteristics. Tea gardens from where samples were collected are situated in the eastern Himalayan region are characterized with the quantity of rainfall, even some experience snowfall [9].

The initial experiments, which were carried out at Makaibari tea experimental garden and Castleton tea estate, both in Darjeeling District under Kurseong Subdivision of West Bengal, gave interesting and thought provoking outcomes. Investigations were carried out for a time period of one year, at monthly interval in Makaibari tea estate, which assured the propagation of rhizosphere microbial populations of relatively new tea plants as primarily expected [10]. On the contrary, already

established tea bushes with rhizosphere was found to hinder growth of microbes as observed in terms of the microbial population in the area infested by rhizosphere and microbial population of non-rhizosphere area [11]. Microbial analyses of samples obtained from the soil of Castleton tea estate, where the bushes were of assarnica type and the plantations were more than twenty years old which also showed strong inhibition of rhizosphere microbial communities [12].

Out of the microbial communities, namely - actinomycetes, bacteria and fungi, bacteria were the maximum subdue group among the matured tea rhizosphere. Fungal and Actinomycetes populations were also suppressed but to a much lower extent. The stimulation of microbes in the rhizosphere ("rhizosphere effect") due to plant roots is a popularly known and normal phenomenon and indicates a "positive" influence of plant roots on rhizosphere microorganisms. It is an important observation and is against the general norm. Similar experiments were also conducted from various tea gardens to justify the status of rhizosphere effect in established bushes [13].

RESULTS AND DISCUSSION

The soil samples were obtained from various locations (1) Rohini tea estate (2) Ambootia tea estate and (3) Goomtee tea estate. The tea bushes were of various age groups. While Rohini and Ambootia tea estates represented the well-maintained tea plantations, Goomtee tea estate was not used for a long time. It has been observed that in most of the cases, the R:S showed same trend as received from Makaibari tea plantations, except in case of estimations of bacterial populations from Rohini tea estate, that showed inhibitory effect even at 4years of age [14]. All the tea gardens under discussion are situated in the Himalayan range representing the subtropical or temperate conditions which are generally characterized by rainfall and or snowfall (*Table 1*).

Table 1 Comparative data of age of tea plants and climate in different study areas

Study Area	Age of tea plants	Altitude in m above mean sea level	Mean monthly temperature in degree Celsius		Total rainfall in millimetre
			Maximum	Minimum	
Rohini Tea Estate	4, 8, >100	1300	25.5	13.5	2400
Ambootia Tea Estate	>30	1400	23.5	13.5	2300
Goomtee Tea Estate	4, 15	1600	20.5	8.5	1800
Makaibari Tea Estate	32,44,123	1400	18.5	14.5	2100
Karbia Tea Estate	>100	1577	23.5	10.5	1100

The 'negative rhizosphere effect was more prominent in sophisticated tea estates rather than abandoned tea estates. This was found from two tea gardens, known as (1) Selim Hill tea estate, where from different quantity of soil samples were taken developing along the borders of the tea estate and (2) St. Mary's tea garden having the detreated. Some recent observations performed from an immature tea estate at Karbia, impact of rhizosphere found to be positive in nature in case of actinomycetes and fungi. Various factors that might be responsible at Selim Hill tea estate in terms of minimizing the population of microbes [15].

CONCLUSION

Tea is the main plantation crop in Kurseong hill area, and the finest qualities of tea are produced here. Tea is one of the tourist attractions of this region. With the

enhancement of eco-tourism, tea-tourism would also play an important role towards more and more revenue generation. Research and developmental activities have been done to estimate in which way the microbes are associated along with the tea plantations in various tea estates. Several thousand lakhs of microbes live in close association with the soil and plants in tea gardens. This is almost similar with the microorganisms inside the human body. Tea-tourism, if appropriately organized, is expected to upgrade the livelihood of workers of tea gardens. Through tea-tourism, visitors to the tea estates will purchase local handicrafts, folk medicines and can have the essence of local cuisine which will further pave the opportunity for more national and international tourists ultimately benefiting local people.

The target is to be able to rebuild the rhizosphere community which is synthetic in nature and can be utilized to enhance water harvesting efficiency, management of nutrients and sustainability towards climate change. Eco-huts, eco-village concept may further be encouraged in the tea estates throughout Kurseong sub-division for further betterment of eco-tourism. With the help of metagenomic techniques several microbial species associated with bulk soil, root endophytes and rhizosphere soil of tea plantations were analysed having a background. From different results and data obtained, it has been observed that almost eighty percent of the tea rhizosphere and fifty four percent of the tea root endophyte were unclassified, and which may be part of unculturable section. As a matter of fact of the inability to predict their metabolic needs, these microbes are unculturable.

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Bio-Nanotechnology and COVID-19

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ABSTRACT

Nanotechnology can solve many health problems caused by the coronavirus pandemic. This study will explore in depth how nanotechnology can help in fighting against this pandemic and the ongoing mitigation strategies. Nanomaterials are currently being developed and marketed for COVID-19 storage, detection and treatment. On the other hand, nanotechnology in various fields of science and technology can be of great help in the diagnosis, prevention as well as treatment of COVID-19.

Key words: Nanotechnology, COVID-19, Nanotherapy, Diagnosis, Health

INTRODUCTION

Outbreak of the global issue pertaining to new deadly virus called the new Coronavirus 2019 (2019-nCoV), the World Health Organization (WHO) announced Coronavirus (COVID-19) an epidemic and a state of emergency in 2019. COVID-19 is said to have come from Wuhan, China. In order to maintain control on disease as well as for the prevention of the same, various centers are available in three different countries around the world, including the United States, Germany, and Vietnam [1].

However, WHO rejected clear evidence prior to January 14, 2020, referring to a report that should also be discussed and examined by the Chinese authorities that the individual's personal transmission occurs before January 14, 2020. To date, the coronavirus has infected more than a million people worldwide and has caused more than 55,000 victims. In most countries, a curfew and a mandatory quarantine have been officially reported, hoping to prevent the virus from spreading too quickly [2].

In general, coronaviruses are a different family of helically coiled RNA viruses that contains the largest 26 to 32 kilobase genomes known among all RNA viruses. They are found in a wide variety of hosts that can infect species such as mammals and birds. Four major types of coronaviruses are α-coronaviruses, β-coronaviruses, ϒ-coronaviruses and ∂-coronaviruses, where α-coronaviruses and β-coronaviruses infect mammals; ϒ-coronaviruses infect birds and

∂-coronaviruses can infect both birds and mammals [3].

The new human zoonotic coronavirus was first reported by the Chinese Centers for Disease Control and Prevention (CCDC) 2 on January 9, 2020. The coronavirus genome is encapsulated in a helical protein coating known as capsid and lipid molecules. In particular, the viral envelope contains three structural proteins, and the viral mechanism contains membrane proteins, including nucleocapsid and envelope proteins [4].

Spike protein in coronaviruses forms corona-like structures, which emerge from the surface of the virus used to penetrate host cells. The S1 and S2 are involved in it, where S1 binds to the receptor and S2 for fusion with membrane of host cells. S protein is common target of neutralization by antibodies and other vaccines. Nucleocapsid protein is virtually unchanged and used as a marker in diagnostic tests [5].

Diagnostic Aspect

Coronavirus Respiratory Disease 2019 (COVID-19) clinical trial includes the use of reverse transcription polymerase chain reaction (rRT-PCR) performed in samples collected by the respiratory system by various methods such as swab from nasopharyngeal routes. The reports are displayed for several hours to 2 days. COVID-19 and SARS-CoV-2-based diagnostic kits were developed by BGI team and the U.S. Centers for Disease Control and Prevention. Coronavirus RNA is converted to DNA, and the multiplier is tracked millions of times until the analyzer, called the PCR tool, finds the corresponding copy of the duplicated DNA. A person is positive for the virus if the virus genetic code is recognized. Most of the newly created sets available are capable of finding different genes within the coronavirus and come with three analyzes. If mutation is there, the complex will be able to find and recognize the new generation formed. For one or two tested positive responses, the results should be written as a catalog of viral genomes to prevent the spread of new viruses [6].

Available complexes can find target proteins called human RNA polymerase protein (ORF1 gene), nucleocapsid protein (N gene), and envelope protein (E gene). There is also a set that corresponds to the other protein (gene S) on the coronavirus. In addition to the complexes, there are currently other detection platforms. Nano sensors will replace heat-sensitive weapons used to detect and measure high heat in

people suspected of infecting airports and borders [7].

Role of Nanotechnology in treatment of COVID-19

Till now, no clear cure or vaccine has been proposed for the treatment of COVID-19. The exception is the drug traditionally used to improve the immune system i.e. chloroquine. However, Nano pharmaceuticals, can not only make a major contribution to medicine and pharmaceuticals, but also emerge to prevent the lethal outbreak of COVID-19 worldwide [8].

Nanoparticles are expected to alter size and enhanced properties as a result of a significant increase in surface area between volume and volume. The structure of the coronavirus reveals a resemblance to nanoparticles. Influence or binding of small nanoscale particles, such as viruses, to the highest levels of proteins is primarily due to the fact that infrared electromagnetic radiation causes the structure of the virus to be disrupted [9].

Theranostics is a new drug that includes the detection and neutralization of viruses with Nano drugs and Nano pharmaceuticals with an emphasis on diagnosis and treatment. As a result, there are reports of the application of nanoparticles to combat the microorganisms that cause influenza and tuberculosis. Due to their potential for surface modification and functionalization, nanoparticles are able to trap pathogens and viruses and numerous reports have been reported [10]. The nanoparticles can also be modified or functionalized to dissolve the viral lipid membrane, bind proteins at the S1 peak, and envelope and encapsulate nucleocapsids and RNA. Nanoparticles can be processed to attack certain pathogens. Given their size, the modified nanoparticles act to detect viruses without causing problems to the body or interfering with other functions that are specifically involved in the human immune system. If COVID-19 lasts longer than this year, we need to tailor the current research strategies to address the major stresses on our healthcare that COVID-19 has created. The Society of Nanotechnology can make an important contribution to the war against COVID-19. Nanomaterials are used in care diagnostics, and vaccine development [11].

People infected with SARS-CoV-2 may be carrier of the same broad spectrum of symptoms as other respiratory infections or silence. COVID-19 co-proliferation is a major concern. It is important to conduct economically viable and rapid diagnostic tests for physicians in local hospitals. With

these diagnoses, frontline employees can simply scan the patient and prevent virus spread. Diagnosis is important in determining the spread of infection. With rapid diagnosis and mass surveillance, public health workers can monitor the spread of the virus, actively identify areas of infection, anticipate an increase in capacity, and direct the necessary resources where necessary [12].

CONCLUSION

The success of this system depends on the open cooperation and communication between the federal government and major health institutions. The WHO argued that broader evidence is needed to prevent the epidemic. Patients may need treatment after identified with COVID-19. These treatments block the viral replication. The main research of Nano biological effects can be adapted to understand how SARS-CoV-2 affects cells. Vaccines are the key to preventing disease by boosting their immunity against pathogens. The life we know before this epidemic will change forever. Our society is capable of accelerating the translation of development and using nanotechnology as a leading tool.

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In-silico based development, identification and characterization of EST based SSR from Cinnamon

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ABSTRACT

Background: The discovery of genes and to construct genome mapping simple sequence repeats (SSR) markers plays very crucial role which were derived from the expressed sequence tags (ESTs). The research objective is to develop EST based SSR markers, in Cinnamon and to study its genes and other aspects. This plant is attribute with high medicinal value. It contains several secondary metabolites and other compounds like alkaloids, steroids. Flavonoids, saponins, proteins, tannins, polyphenols, and glycosides, known to impart specific functions and hold important therapeutic roles.

Results: A total 7331 EST sequences of Cinnamon, were retrieved from the dbEST database in FASTA format. Among, these 1139 SSRs were identified, which includes 692 repeat unit for mononucleotides repeats, 161 repeat units for dinucleotide repeats, 270 repeat units for trinucleotide repeats, 11 repeat units for tetra nucleotide repeats, 1 repeat unit for penta-nucleotide repeats, and 4 repeat units for hexa-nucleotides repeats. Following identification of SSR, BLAST (Basic Local Alignment Search Tool) alignment of EST SSR was performed, which corroborated their functions. However, most of them were attributed to essential protein and many were gene related proteins, some of them were metabolically active proteins and enzymes were identified in the plant.

Conclusions: Findings will helps to analysis the important molecular markers and to facilitate the analysis of genetic diversity.

Keywords: Expressed sequence tags-simple sequence repeats, *in silico* studies, functional markers, cinnamon.

INTRODUCTION

Cinnamon or known as 'true cinnamon' is native to Sri Lanka and southern parts of the India. Cinnamaldehyde, eugenol, and linalool are the three main components of the essential oils obtained from the bark of Cinnamon, these components represent 82.5% of the total composition [1]. Trans cinnamaldehyde, accounts for approximately 49.9-62.8% of the total amount of bark oil [2,3]. Two more major components of cinnamon extracts are cinnamaldehyde and eugenol [4]. There are two main verities of cinnamon, *Cinnamomum zeylanicum* (CZ) and *Cinnamon cassia* (CC). These verities have basic difference in their coumarin (1,2-benzopyrone) content [5]. The levels of coumarins in CC seem to be very high and can cause health risk if consumed regularly in higher quantities. According to the German Federal Institute for Risk Assessment (BFR), 1 kg of CC powder contains approximately 2.1-4.4 g of coumarin, which means 1 teaspoon of CC powder would contain around 5.8-12.1 mg of coumarin. Above given is the TDI (Tolerable Daily Intake) for coumarin if 0.1 mg/kg body weight/day which was recommended by the European Food Safety Authority (EFSA) [6]. The BFR reports precisely states that CZ contains 'hardly any' coumarin. Coumarins are secondary phyto-chemicals with strong anticoagulant, carcinogenic and having hepato-toxic properties [6]. The fundamental mechanisms for the coumarin content-related toxic effects are yet to be completely clarified. CC contains high concentration of coumarin than any other foods. Studies have shown that coumarin coverage from food consumption is mainly due to CC. Currently available evidences shown that coumarin does not appear to play any direct role in the observed biological effects of CC. However, CC variety has been shown many beneficial pharmaceutical properties [6,7]. Numerous beneficial health effects of CZ have been confirmed through *in-vitro* and *in-vivo* studies in animals. They have anti-inflammatory properties, reducing cardiovascular disease, anti-microbial activity, boosting cognitive function and reducing risk of colonic cancer. Cinnamon has been also mentioned in chinese texts as long as 4,000 years ago, it is one of the oldest herbal medicines known [8].

EST-SSR (expressed sequence tag-simple sequence repeat) is a new developed molecular marker based on the expression sequence of microsatellites. This technology has attained the advantage of avoiding the

construction steps of genomic DNA library in SSR development process; it gives the exact marker involve in gene function and shows similarity in genomic functional area. EST-SSR explains the phenotypic difference based on its polymorphisms. This EST-SSR are highly conserved within the species as it is a part of gene which leads to make the primers more commonly used among the species. Therefore for the development of SSR markers, these EST sequences act as valuable resources. In the recent years, several studies revealed that there are vast numbers of ESTs accumulated as the result of deep research analysis on different species. These accumulated EST data provides a platform in the development of SSR markers [9-11].

Various projects on sequencing or ESTs generates large amount of DNA sequence data which can be easily accessible to public, it carries both genic (EST) and genomic sequences which can be further used in the development of markers such as SSRs, SNPs. etc. The presence of any marker type from such data which can easily accessible leads to the generation of markers in cheap cost, like if SSRs are present in the genic sequence, they called as EST-SSRs [9].

The EST-SSR markers are associated with the genes carrying them as once they mapped. They also act as a valuable source of functional markers. Thus the formation of EST based SSR markers is a cheap alternative as compared to conventional SSR development method. In genome analysis of sorghum these EST-SSRs play a major role in producing lasting insight into processes by which novel genotypes are generated, such advantages helps in the applications of crop breeding programs [9-11].

The Conventional method of the development of SSR marker is tedious and costly. Therefore, the availability of genic EST sequence or genomic sequence in open public databases and availability of bioinformatics tools, the development of SSR marker is becoming now low cost and easier [12]. Although, previously several SSR markers were already generated by using EST databases in several crops. For the diversity analysis the EST-SSR markers were widely used in several crops like: wheat [10,13,14], barley [9], in mapping of barley [11, 15], pearl millet [16] and finger millet [17]. The genomic SSRs derived from the transcribed regions of the genome are more polymorphic as compare to the EST derived SSR markers [15, 12].

In the terms of cross-species transferability the EST-SSR markers are very superior, because they were derived from the most conserved regions of the genome which are very useful in the application of comparative genome mapping and phylogenetic analysis. EST-SSR markers developed in a small number (30) in sorghum with wheat, rice and maize [18]. These markers have also shown very transfer rate in several crops system. In wheat the EST-SSR markers developed showing 62% transferability across the all four species barley, maize, wheat and rice. EST-SSRs showing 40% transferability rate from barley to rice [11, 15].

MATERIALS AND METHODS

Development of EST-SSR markers: In the improvisation of species, molecular markers are prominently used, they help to identify the polymorphisms, mating system parameters, marker-assisted selection and genotype characterization. Finally EST-SSR was constructed for cinnamon as we found there was no EST-SSR developed till date.

Recognition of EST sequences

Firstly, the EST sequences of cinnamon were retrieved in FASTA format from the NCBI https://www.ncbi.nlm.nih.gov/) i.e. National Centre for Biotechnology Information advances science & health by providing access to biomedical & genomic information. After that the MISA web (http://webblast.ipk-gatersleben.de/misa/) was used for the recognition and determination of the ideal microsatellite also compound microsatellites which are fitful by the certain number of bases from the ESTs recognized from the NCBI followed to design the primers at microsatellite loci.

MISA

In the plant genetics and the forensic science the microsatellites are prominently used marker system. The challenge is to make microsatellites from re-sequencing data. MISA is a web based computational application tool which help in the development of microsatellite markers. MISA web can be accessed by this link http://misaweb.ipk-gatersleben.de/. A 25 years ago microsatellites were rise and still it was a most common genetic marker using in plant breeding and plant genetics and forensics science, where it is generally known as simple sequence repeats (SSRs) or

short tandem repeats (STRs). In the microsatellite the basic structural block is the short sequence motifs present between one and six pairs in length which is repeated in tandem, by high throughput sequencing data or Sanger method these characteristics can be easily detected by giving *in-silico* approach using nucleotide sequences [10,12].

Pre-processing of the FASTA sequences

The retrieved FASTA sequence was pre-processed first by the help of software named CAP3 (http://doua.prabi.fr/software/cap3) which was freely available on web server, it identify the non-redundant EST sequences. The CAP3 software runs algorithm which overlaps between the sequences and further join the reads in the decreasing order to form contigs. After the pre-processing of FASTA sequences CAP3 gave two files ie. Contigs and Single tone which was further processed separately [12].

Selection of candidate EST sequences

The non-repeated SSR containing EST sequences of Cinnamon were used for homology search by using Basic Local Alignment Search Tool (BLAST) tool available in the NCBI. From all the BLAST hits we identified an appropriate EST giving the maximum score was selected, followed by recognition of homologous genomic region. For the analysis of complete coverage across the genome sequence BLAST were performed.

Primer Designing

The selected contigs (SSR containing ESTs sequences) and the single tone were used to design primer pairs by using primer3 (http://biotools.umassmed.edu/bioapps/primer3_www.cgi). The Primers were designed in such a way that they follow such conditions: primer length (min-70nt, opt-160nt, max- 250nt), Tm (min-54°C, opt-57°C, max-60°C) & GC content (min-45%, opt-50%, max- 60%) [12].

BLAST

BLAST is a most common local alignment tool (Basic Local Alignment Search Tool) founded by Altschul. It is based on a set of algorithms in which a fragment of query sequence that aligns with the fragment of subject sequence present in the database. The initial alignment should be greater than neighbour score threshold (T). The alignments can be extended in both the direction till the score aligned segment is increase.

There were two alignments global and local. The global sequence approaches are used to compare the whole sequence with the other full sequences. In the local method the part of the sequence is align with the other part of the sequence. The global alignment gives comparison of one to other sequence, local alignment shows higher similarity in the regions but lack the ability of comparison of two sequences. While comparing small group of sequence global approach is very useful as the comparison of sequences increases the cost increases. The local alignments are based on heuristic programming approach that is very suitable for very large databases, but they do not provide give optimum solution. This limitation plays a major role in the genomics as they uncover regions of similarity that are correlated by two diverse sequences.

SWISS MODEL - ExPASy

ExPASy is the bioinformatics resource portal which gives a key to open scientific databases and software tools in other aspects of life sciences. It carries some useful tools like SWISS MODEL, UNIPROT, PROSITE and STRINGS WISS-MODEL it is a fully developed protein structure homology modelling server access by the ExPASy web server. This server is used to make protein modelling accessible to the all researchers of life sciences worldwide; on the basis of FASTA sequence it provides the 3D structure of proteins.

RESULTS AND DISSCUSSION

MISA (Microsatellite Identification Search Tool)

The EST sequence which retrieve from the NCBI database, the CAP3, and MISA software is used for cinnamon plant, MISA gave the following results as discussed below:-

Distribution frequency of repeat units for all the SSR's in Cinnamon

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RESULTS OF MICROSATELLITE SEARCH
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Total number of sequences examined:                7
Total size of examined sequences (bp):             5
Total number of identified SSRs:                   1
Number of SSR containing sequences:                8
Number of sequences containing more than 1 SSR:    1
Number of SSRs present in compound formation:      2


Distribution to different repeat type classes
---------------------------------------------

Unit size   Number of SSRs
1     692
2     161
3     270
4     11
5     1
6     4
```

Based on the results obtained from the cinnamon MISA analysis, In total 1139 SSRs were identified: out of which 692 repeat unit were mononucleotides repeats, 161 repeat unit for dinucleotide repeats, 270 repeat unit for trinucleotide repeats, 11 repeat unit for tetra nucleotide repeats, 1 repeat unit for penta-nucleotide repeats, and 4 repeat unit for hexa-nucleotides repeats.

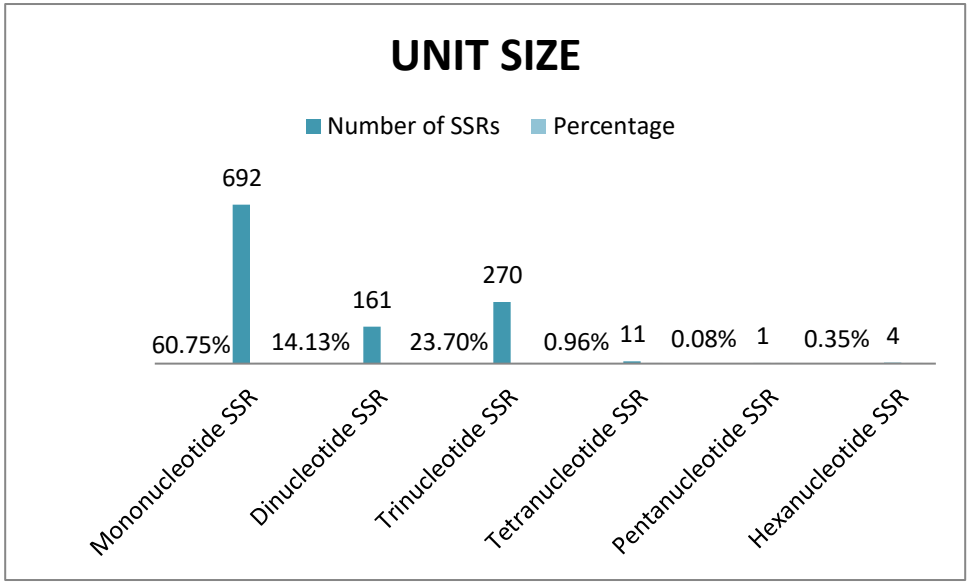


Figure1. Distribution frequency of repeat units for all the SSRs in Cinnamon.

Repeats	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
A/T	-	-	-	-	-	146	106	53	41	31	32	43	25	34	26	13	3	4	7	2	7
C/G	-	-	-	-	-	39	18	12	10	6	3	9	3	2	2		1				1
AC/GT	-	15	10	6	2	2						1									
AG/CT	-	24	5	4	2	6	1	1	3		6	1	4	1			2			1	1
AT/AT	-	13	7	15	6	7	2		1		1	6	1							1	
AAC/GTT				2																	
AAG/CTT	8	3		5																	
AAT/ATT	5	12	8	11	20	3			1												17
ACC/GGT	18	3		1																	
ACT/AGT	3		1																		
AGC/CTG	11	2	3	1																	
AGG/CCT	22	12			1																
ATC/ATG	54	2																			
CCG/CGG	11	26	4																		
AAAT/ATTT	6	3																			
ACAT/ATGT		1																			
AGGC/CCTG	1																				
AAAGC/CTTTG	1																				
AAGCAG/CTGCTT		3																			
ACCATG/ATGGTC	1																				

Figure 2: Distribution frequency of nucleotides for all the SSRs in Cinnamon.

The figure 2 indicated that A/T mononucleotide was the most common repeat among all SSR motifs, while the Most common dinucleotide motif was AG/CT. Among the tri nucleotide repeats AAT/ATT was most common; in tetranucleotide SSR motifs AAAT/ATTT was most common. The pentanucleotide motif was AAAGC/CTTTG, in hexanucleotide SSR motif AAGCAG/CTGCTT was common. These results were similar to previous studies [12].

CAP3

Pre-processing of the EST sequences downloaded from the public domain were carried out by CAP3 software. By using the Cap3 program, which helps in the elimination of repeating data set from the sequence file, ultimately it results into the formation of two files with one containing contig sequence whereas in other the single tone sequence. As summary discussed below:-

Numbers of Contigs: 2233

Number of Single tone: 2305

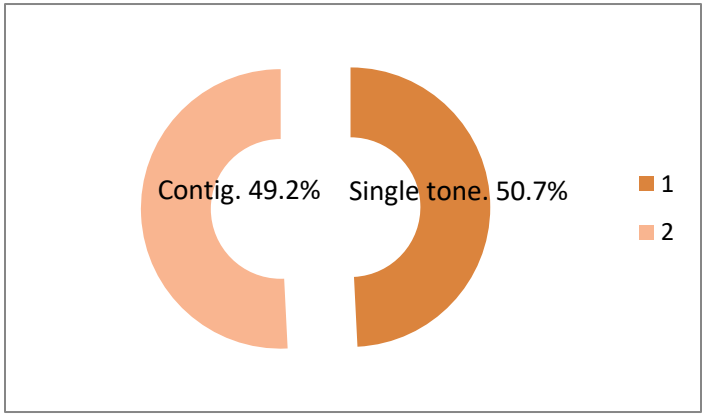


Figure 3: Distribution of single tone & contig in Cinnamon.

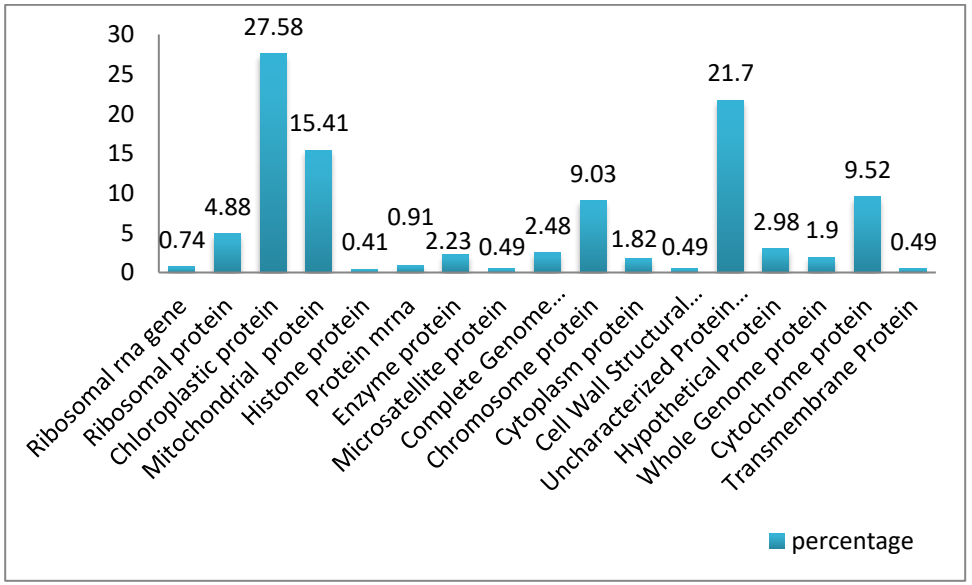


Figure 4: Biological distribution of contig and single tone.

BLAST

BLAST was carriedout by BLAST nucleotide analysis; all the 2233 Contig and 2305 Single tone sequences of Cinnamon were BLAST to analyze the putative function of the sequence. On the basis of their appropriate match, all SSR loci were divided into three groups;

Biological Function: In biological functions the genes acquire all vital processes like metabolism, photosynthesis, cell signalling, environmental related factors, etc. In this analysis we have got 1207 total sequences from Contig and Single tone from the Cap3 software there were found to be 9 Ribosomal RNA gene functionality, 59 Ribosomal Protein, 11 Protein mRNA, 333 chloroplastic & 186 mitochondrial proteins, 5 histone, 27 enzyme, 30 complete genome, 109 chromosome, 6 microsatellite, 22 cytoplasm, 6 cell wall structural, 262

uncharacterized protein, 36 hypothetical protein, 23 whole genome, 115 cytochome & 6 transmembrane protein after the analysis.

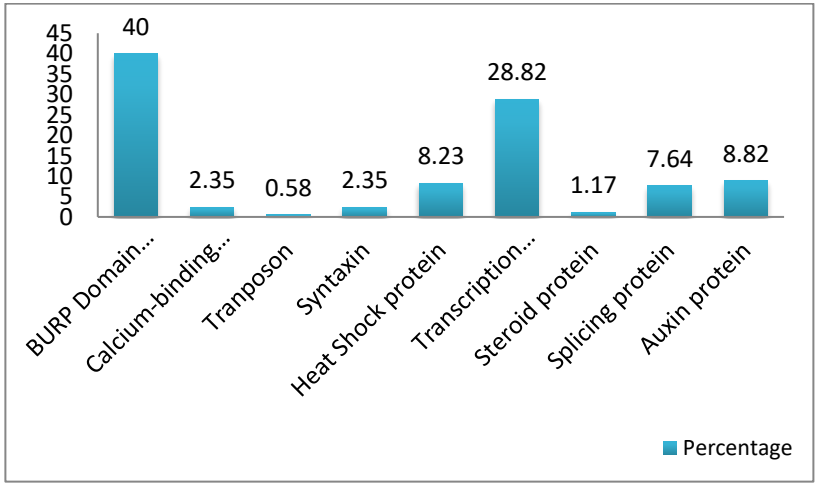


Figure 5: Protein distribution of contig and single tone.

Protein Function: By the help of nucleotide BLAST analysis, there were in total 170 proteins found in the sequences, among these 16 were heat shock proteins, 49 transcription proteins, 15 auxin, 2 steroid, 13 splicing, 68 BURP domain protein, 4 syntaxin, 4 calcium binding protein and 1 transposon.

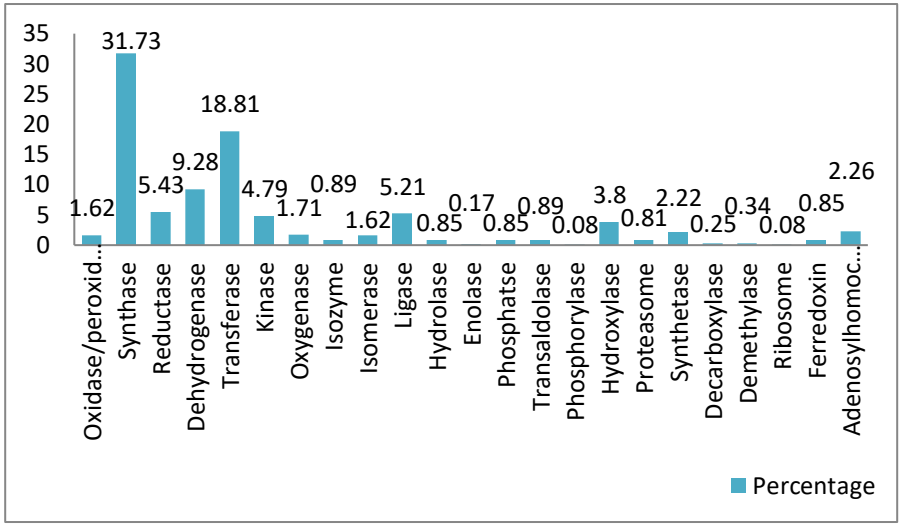


Figure 6: Enzyme distribution of contig and single tone.

Enzymatic Function: Cinnamon is important medicinal plant used for treating various diseases. It contains more than 2338 enzymatic activity with 742 synthases, 127 reductase, 38 oxidases & peroxidases, 21 isozymes, 38 isomerases, 40 oxygenase, 217 dehydrogenase, 112 kinase, 565 transferases, 122 ligase, 20 hydrolase, 4 enolase, 20 phosphatase, 21 transaldolase, 2 phosphorylase, 89 hydroxylase, 19 proteasome, 52 synthetase, 6 decarboxylase, 8 demethylase, 2 ribosome, 20 ferrodoxin and 53 adenosylhomocysteinase.

Primer3

Primers were constructed based on suitable nucleotide and appropriate sequences after BLAST of contigs and single tone.

Table1: Characteristics of EST-derived SSRs for Cinnamon.

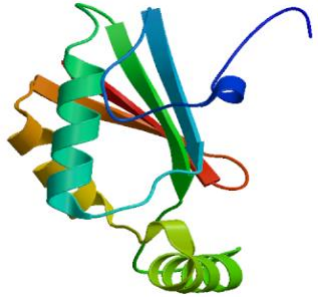
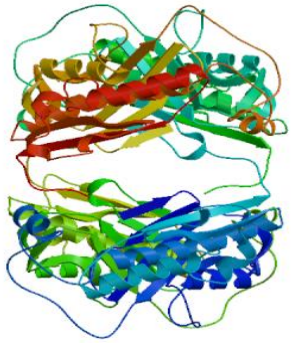
ID	TM (oC)	GC%	Forward Primer	Reverse Primer	Product size	Predicted function based on blast	Accession
DY327125.1	60	50	GCACCATCTTC GTCCTTCAT	TAACATTCCCC AGCTTCGTC	170	Erythrantheguttatus G-type lectin S-receptor-like serine/threonine-protein kinase At1g34300 (LOC105974395), mRNA	XM_012999490.1
DY327131.1	60	50	ATCCTCTGGAA GAGCTGCAA	TGATCAAGTG CGACCTTCAG	234	Erythrantheguttatus pyruvate kinase, cytosolic isozyme (LOC105956735), mRNA	XM_012980626.1
DY327152.1	60	53	ACTCATCTCGA CAGCCTCGT	CGGCACATCTT TCAGGAGAT	207	Agastache rugosa chalcone synthase (CHS) mRNA, complete cds	JQ314450.1
DY327176.1	60	45	CCTTGGTTTTA ACGCTGGAA	GCCATGGGAT AGAGCAAAAA	249	Ocimumbasilicum germacrene D synthase (GDS) mRNA, complete cds	AY693644.1
DY327188.1	59	50	CGCACTCTTCA TCACTCCAA	ACTGCTATAA GCGCCATCGT	249	Sesamum indicum (RS)-norcoclaurine 6-O-methyltransferase-like (LOC105173010), mRNA	XM_011094646.2
DY327192.1	59	53	ACTGTTGGACC ATCCAGAGG	CCCAAAGCAA GAATCTCAGC	155	Sesamum indicum cyclin-dependent kinase D-3 (LOC105166737), transcript variant X2, mRNA	XM_020695628.1
DY327215.1	60	48	CCACTTCATGC TCCCTGTTT	GAAGCAAAAT TCGGTTGGAA	234	Sesamum indicum 3-phosphoshikimate 1-carboxyvinyltransferase 2 (LOC105171218), mRNA	XM_011092260.2
DY327278.1	60	50	ATGAGAAACA TGGCGAGGAC	TTCTTCTTCTC AGCGCCTTC	208	Sesamum indicum protein SRC1 (LOC105173924), mRNA	XM_011095843.2
DY327305.1	60	53	GAAGGACTTC	TGCTTAACAGC	162	Sesamum indicum serine/threonine-protein	XM_011088543.2

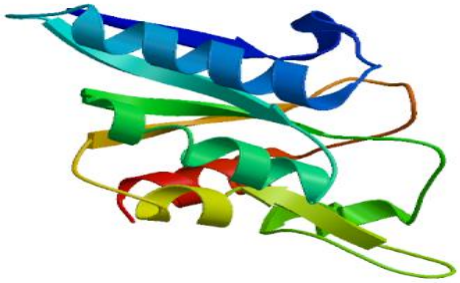
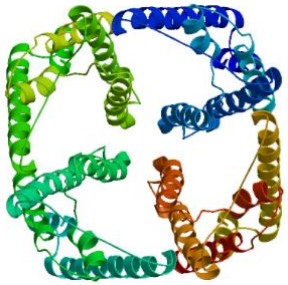
			CCCGATTCTC	AACGACCTG		kinase PBS1 (LOC105168454), mRNA	
DY327320.1	60	50	AGAGAGAGAT TCGCCGATCA	TTCGTCACTCG TGCTGAAAG	219	Olea europaea var. sylvestris serine/arginine-rich splicing factor SR45a-like (LOC111369703), transcript variant X3, misc_RNA	XR_002698229.1
DY327324.1	60	50	ATCCCATCCAT CCTTCCTTC	CGATCGACAC ATCGAAGCTA	155	Sesamum indicum glycosyltransferase family protein 64 protein C5 (LOC105174171), mRNA	XM_011096181.2
DY327360.1	60	50	AAACACAAGG TGCACCACAA	GCGATGGAGA GCCAACTTAG	180	Sesamum indicum autophagy-related protein 18f (LOC105176725), mRNA	XM_011099623.2
DY327460.1	60	50	CCTTGGTTTTA ACGCTGGAA	GCCATGGGAT AGAGCAAAAA	249	Ocimumbasilicum germacrene D synthase (GDS) mRNA, complete cds	AY693644.1
DY327475.1	59	50	CAAGCTGTTCA ACCCCAAAT	AGCGAGCTTC CTCATCTCAG	178	Sesamum indicum acyl-coenzyme A oxidase 3, peroxisomal (LOC105178460), mRNA	XM_011101927.2
DY327481.1	60	50	GCAAGGTAGT GCCCAATCAT	GAAGTTGCGC AAGGCTAAAC	177	Sesamum indicum 40S ribosomal protein S15 (LOC105171649), mRNA	XM_011092832.2
DY327482.1	59	55	ATCATTTGTGG AGGGAGTGC	CCCTTGACCCC CTTAGACTC	199	Erythrantheguttatus serine hydroxymethyltransferase 4 (LOC105964521), transcript variant X2, mRNA	XM_012989028.1
DY327495.1	60	50	AGTGATCTCTT TGGGCATGG	TGAGAGCAAG GGAGGAGAAA	166	Ocimumbasilicum gamma-cadinene synthase (CDS) mRNA, complete cds	AY693645.1
DY327503.1	60	50	GAGGTCGAAG ATCCCACAGA	TCAAATTGGTG CTCTTGCTG	176	Sesamum indicum serine hydroxymethyltransferase 4 (LOC105166533), mRNA	XM_011085916.2
DY327504.1	59	55	ATCATTTGTGG AGGGAGTGC	CCCTTGGACCC CTTAGACTC	199	Erythrantheguttatus serine hydroxymethyltransferase 4 (LOC105964521), transcript variant X2, mRNA	XM_012989028.1

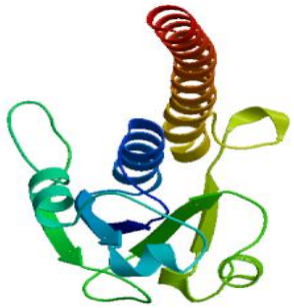
SWISS MODEL-EXPASY

The 3D structures of important protein which were represented by contings and single tome sequences developed with the help of SWISS Model Expasy.

Table 2: Proteins Structures predicted on the basis of BLAST results.

PREDICTED: Sesamum indicum (RS)-norcoclaurine 6-O-methyltransferase-like (LOC105173010), mRNA	
PREDICTED: Sesamum indicumchorismate synthase 1, chloroplastic (LOC105166625), mRNA	

PREDICTED: Erythrantheguttatus protein BPS1, chloroplastic-like (LOC105960562), transcript variant X2, mRNA	
PREDICTED: Sesamum indicum autophagy-related protein 18f (LOC105176725), mRNA	
PREDICTED: Sesamum indicum aquaporin TIP1-1-like (LOC105169946), mRNA	

PREDICTED: Sesamum indicum syntaxin-112-like (LOC105178362), mRNA	

CONCLUSION

In the present, developed EST-SSR will be highly useful in genotyping of cinnamon accessions with microsatellite markers, that can reveal the genetic diversity among accessions. These information will help us to select better parents with desired genes for the progeny to develop new commercial variety. It helps to generate novelty of the species with higher productivity and quality traits towards the sustainable development. The development of cinnamon SSR further helps in characterization of potential genetic makers which are very important for crop improvement and in gene mapping. These EST-SSR markers play a major role in determining the genetic relationship, pedigree analysis and genetic background of the species.

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