EFFECT OF CULTURAL CONDITION ON EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF METHANOLIC BARK EXTRACT OF *ANOGEISSUS LATIFOLIA* ON ETHANOL-INDUCED HEPATOTOXICITY

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ABSTRACT

Objective: Geographical location has a great importance on phytoconstituents content in plants. The present study was aimed to understand the impact of soil nature of geographical zone on evaluation of hepatoprotective activity *Anogeissus latifolia* (AL), (family: Combretaceae) methanolic bark extract on ethanol-induced hepatotoxicity in Wistar rats.

Methods: Soil sample was collected and analyzed for various physicochemical properties. Atomic absorption spectrophotometer was used for determination of heavy metals present in soil sample. Thereafter, liver damage was produced by ethanol (3.76 g/kg p.o). The plant extract was administered orally for 25 days. At the end of the study, the biochemical parameters were investigated. Silymarin was used as a standard. Furthermore, histopathology was carried out and characterized by severe vacuolar, cytoplasmic degeneration, and hepatic necrosis. Finally, the impact of soil parameters is correlated with the evaluated study.

Results: Analyzed soil sample showed pH is acidic (pH=5.48) and presence of higher amount of iron (Fe), copper (Cu), and zinc (Zn) (77.37 mg/kg, 10.87 mg/kg, and 19.26 mg/kg, respectively) whereas other metals such as nickel, chromium, cadmium, and lead are present in less content. Thereafter, treatment of animals with methanolic extract of AL bark significantly (p<0.05) decrease the levels of serum glutamic oxaloacetic transaminase in serum which is an indicative of hepatoprotective activity. Also brought down the level of serum glutamic pyruvic transaminase significantly (p<0.05 in 200 mg/kg b.w. and 400 mg/kg b.w. doses, respectively). Various parameters physical parameters, cholesterol level, and total protein were estimated. Histopathology studies and triglycerides also were indicative of hepatoprotective property.

Conclusion: Finally, the results concluded that the presence of higher amount of Fe and Zn helps to get the significant results of the present investigation which has great impact on liver protection and the selected plant extract showed dose-dependent significant hepatoprotective effect against ethanol-induced hepatotoxicity in Wistar rats.

Keywords: *Anogeissus latifolia*, Atomic absorption spectrophotometer, Biochemical parameter, Ethanol, Hepatoprotective, Metals, Silymarin, Soil sample.

INTRODUCTION

Traditionally, plants are used to treat many diseases and are a good source of novel drug compounds. Plant-derived medicines have made a large contribution to human health. In that metabolism and excretion are the basic and important functions which are maintained by the liver. But sometimes distortion of metabolic functions occurs due to any causes among that hepatic injury is the major one and it causes serious health problems [1]. Drug-induced liver injury is a major health issue that challenges to the health-care professionals as well as to the Indian Pharmaceutical Industries. Hence, alternate herbal treatment is the recent focus toward the liver protection, though the herbal treatment is slower than synthetic medicines due to less side effects and route level curing of diseases, it is accepted in medical sciences. Medicinal plants or their bioactive compounds have been utilized for hepatoprotective activity by the developing countries for primary and traditional health-care system since very long period of time [2]. There are many plants which scientifically revealed to have hepatoprotective activity [3-8].

Oflate, *Anogeissus latifolia* (AL) is a medicinal plant belongs to family Combretaceae, having various medicinal properties. It is a large or moderate-sized tree available throughout India. The plant is traditionally used for the treatment of dysentery, snakebite, leprosy, diabetes, wounds liver damage, ulcers, and skin diseases [9,10]. Various secondary metabolites, namely, tannins, gallic acid, ellagic acid, and flavonoids such as lutin and quercetin are identified which acts as potential antioxidant [11,12]. There are few reports on hepatoprotective activity [13,14] of the plant bark, but no reports on correlation of soil with the said activity. Hence, the present study was planned to correlate the impact of soil conditions on therapeutic activity and also reaffirm the effect of AL bark on ethanol-induced hepatotoxicity in Wistar rats.

METHODS

Plant collection
The bark of AL used for the present studies was collected from Ooty district of Tamil Nadu during August end in the year 2016. The bark was identified and authenticated by comparing with voucher specimen available at Survey of medicinal plants and collection unit, Department of AYUSH, Ministry of Health and Family Welfare, Government of India, Emerald by Field Botanist Dr. Rajan S. A voucher specimen (No: PRIST/Bark-AL/PB/2017) was also kept in laboratory for future reference.

Soil sample analysis
Soil samples were collected at the depth of 6–12 cm. Then, the pH was measured with soil pH meter, using equal ratio of soil and water and electrical conductivity was measured with a electroconductometer and probe as using a 1:5 soil and water ratio [15]. The percentages

of sand, silt, and clay was determined with International pipette method [16]. Cation exchange capacity was determined in $BaCl_2$ by the Gilman method [17] and total organic carbon (C) was determined by wet dichromate oxidation method [18]. Thereafter, total metals (iron [Fe], copper [Cu], zinc [Zn], lead, cadmium, nickel, arsenic, and chromium) were determined with the help of atomic absorption spectrophotometer (AAS) (AAS, Perkin Elmer model: AAnalyst 100; Australia) by acid digestion method.

Extraction of bark sample

Bark sample was size reduced using Hammer mills and then 500 g of sample was extracted using methanol as solvent by reflux method for 8–10 h. After extraction, the solvent was filtered with Whatman filter paper (No.1) and further concentrated using water bath to get the extract. The percentage yield of the methanolic extract of AL (MEAL) was calculated and kept in airtight glass bottle in refrigeration condition at 5°C for further investigation.

Phytochemical screening

Extracted bark sample further screened for the presence of various phytoconstituents as per the standard method [19,20]. For the entire tests, 1 ml of the extracted sample was used for the detection of specific color and the result was tabulated in result section.

Experimental animals

Wistar rats of either sex weighing between 150 and 200 g were used in the present study. They were allowed free access to a standard pellet diet and water *ad libitum* and were maintained under standard laboratory conditions with alternating light and dark cycles of 12 h each at 25±2°C. They were acclimatized to laboratory conditions for 5 days before behavioral studies. All the readings were taken during the same time of the day, that is, between 8 a.m. and 11 a.m. The Institution Animals Ethics Committee had approved the experimental protocol (No:1611/PO/a/12/CPCSEA) and care of animals was taken as per the guidelines of CPCSEA, Department of Animal Welfare, and Government of India.

Grouping of animals and experimental protocol

Wistar rats were divided into five groups and each group consists of six rats. The experimental design is shown in Table 1.

Histopathological examination

On 26th day, the animals were then anesthetized using desiccator containing excess carbon dioxide and blood samples were collected by retro-orbital puncture and biochemical parameters such as alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), triglycerides (Tri), cholesterol (Ch), total proteins (TPs), and albumin were estimated. Livers from all animals were removed. Small piece of liver tissue was collected and preserved in 10% formalin solution for histopathological studies. A paraffin embedding technique was carried out and sections were taken at 5-mm thickness, stained with hematoxylin and eosin, and examined microscopically for histopathological changes [21].

Correlation study

Metal ion contents were correlated with the various biochemical parameters with two-tailed analysis (p value) and computed "r" for every pair of data sets. Further catalase (CAT), superoxide dismutase (SOD), and lipid peroxidation (LPO) were also correlated with metal ion contents and showed positive significant results.

Statistical analysis

The results were analyzed as standard error of mean (SEM) for each group (mean ± SEM). All groups were subjected to one-way analysis of variance followed by Dunnet's *post hoc* test and p<0.05 was considered as statistical significant.

RESULTS

Analysis of soil samples

Soil sample was analyzed and various physiochemical parameters were tabulated in Table 2.

Yield of extracts

Percentage yield of the extract was calculated by following formula and procured maximum yield of 20.89% (w/w) which was sufficient for the targeted experiment.

$$\% \text{ yield } = \frac{\text{weight of the extract}}{\text{weight of the raw material}} \times 100$$

Table 1: Grouping of animals

Groups	Treatments
A	Normal control (only distilled water)
B	Ethanol (3.76 g/kg, p.o)
C	Ethanol intoxicated animals+standard drug, silymarin (200 mg/kg p.o)
D	Ethanol intoxicated animals+MEAL (200 mg/ml b.w)
E	Ethanol intoxicated animals+MEAL (400 mg/ml b.w)

MEAL: Methanolic extract of *Anogeissus latifolia*

Table 2: Soil parameter test

Parameters	Soil sample of Ooty, TN
pH	5.48±0.20
EC (1:5) (m/Scm)	11.94±0.01
Texture	Loose lateritic loam soil
CEC (cmol/kg)	15.03±0.10
Total metal content (mg/kg)	
Fe	77.37±0.10
Cu	10.87±0.22
Zn	19.26±0.11
Ni	5.33±0.01
Cd	3.77±0.21
As	1.02±0.02
Pb	6.28±0.23
Cr	2.11±0.12

EC: Electrical conductivity, CEC: Cation exchange capacity, Fe: Iron, Cu: Copper, Zn: Zinc, Ni: Nickel, Cd: Cadmium, As: Arsenic, Pb: Lead, Cr: Chromium

Table 3: Details of qualitative phytochemical tests

Sr. No	Test	Methanolic Extract
1	Carbohydrates	
	Mohlish's test	Positive
	Fehling's test	Negative
2	Proteins and amino acids	
	Ninhydrin test	Negative
	Biuret test	Negative
3	Alkaloids	
	Mayer's test	Positive
	Wagner's test	Positive
4	Fixed oils and fats	
	Spot test	Negative
5	Glycosides	
	Borntrager's test	Positive
	Legals test	Positive
6	Triterpenoids	
	Tin+thionyl chloride	Negative
7	Phenolics and tannins	
	Ferric chloride test	Positive
	Lead acetate test	Negative
	Dilute HNO3 test	Negative
8	Saponins	
	Foam test	Positive
	Hemolysis test	Positive
9	Flavones and flavonoids	
	Caddy's test	Positive
	Shinoda test	Positive

Phytochemical screening

Details phytochemical screening was carried out with the help of documented procedures of chemical tests and revealed the presence of carbohydrate, alkaloids, glycosides, tannins, saponins, and flavonoids. The overall result was tabulated in Table 3.

Animal experiment for hepatoprotective

There was increase in levels of serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), TP content, ALP, LPO Ch, and Tri significantly, which indicates the necrosis of hepatic cells. The rats treated with extract showed significant reduction in all the biochemical parameters elevated by ethanol. The reduction in the values of biochemical parameters is given in Tables 4-6.

Histopathological studies

The histopathological evaluation of ethanol toxicity in all the groups was examined and shown in Fig. 1. The study showed that section of rat liver treated with vehicle control group showed liver parenchyma with intact architecture which is the normal appearance. Section of the liver in toxicant control group shows partially affected architecture. Some of the hepatocytes showed apoptotic changes, perivenular mononuclear inflammatory infiltration, scattered inflammatory infiltration within the parenchyma which is due to toxicity. Section of the liver in silymarin-treated group showed sliver parenchyma with intact architecture. Some of the central veins show congestion with diffuse congestion of sinusoids. Section of liver in test drug-treated groups (200 and 400 mg/kg) showed intact architecture, few regenerative hepatocytes, sinusoidal congestion, and scattered mononuclear inflammatory cells which is similar to silymarin-treated group.

Correlation study

Metal ion contents, especially Fe, Cu, and Zn were correlated with the various biochemical parameters (at confidence interval 95%) and the results were tabulated in Table 7.

Further correlated with the CAT, SOD, and LPO activities and depicted in Table 8.

Table 4: Effect of MEAL bark on SGPT, SGOT, and ALP levels in ethanol-induced hepatotoxic rats (n=6)

Group	Treatment	Dose	SGPT (U/L)	SGOT (U/L)	ALP (ml/100 g)
A	Normal control	10 ml/kg p.o	28.35±0.90	33.90±1.50	27.15±1.14
B	Toxicant control	Ethanol 3.76 mg/kg, p.o.	122.9±1.50	168.26±1.35	94.21±1.42
C	Standard	200 mg/kg, p.o+Ethanol	33.27±0.05**	64.21±0.12**	32.36±2.05**
D	MEAL	200 mg/kg, p.o+Ethanol	36.78±0.05**	68.32±0.50**	40.12±0.11**
E	MEAL	400 mg/kg, p.o+Ethanol	34.12±0.05**	66.22±0.50**	34.40±0.07**

Values are mean±SEM (n=6) one-way ANOVA, compared with the Group B; **$p<0.05$ is consider as statistical significant (n=6). MEAL: Methanolic extract of *Anogeissus latifolia*, SGPT: Serum glutamic pyruvic transaminase, SGOT: Serum glutamic oxaloacetic transaminase, ALP: Alkaline phosphatase, SEM: Standard error of mean

Table 5: Effect of MEAL bark on serum total protein, total cholesterol, and triglyceride levels in ethanol-induced hepatotoxic rats

Group	Treatment	Dose	Total protein levels (g/dl)	Cholesterol levels (mg/dl)	Triglyceride levels (mg/dl)
A	Normal control	10 ml/kg p.o	6.90±0.05	6.39±0.165	28.25±0.453
B	Toxicant control	Ethanol 3.76 mg/kg, p.o.	32.18±0.06	68.03±0.543	87.20±2.53
C	Standard	200 mg/kg, p.o+Ethanol	5.72±0.16**	9.00±0.40**	18.71±0.15**
D	MEAL	200 mg/kg, p.o+Ethanol	4.05±0.15*	12.03±0.12*	21.08±0.12*
E	MEAL	400 mg/kg, p.o+Ethanol	6.10±0.08**	10.19±0.01**	19.30±0.11**

Values are mean±SEM (n=6) one-way ANOVA, compared with the Group B; **$p<0.05$ is consider as statistical significant (n=6). MEAL: Methanolic extract of *Anogeissus latifolia*, SEM: Standard error of mean

Table 6: Effect of MEAL bark on CAT, SOD, and LPO in ethanol-induced hepatotoxic rats

Group	Treatment	Dose	CAT (U/mg of protein)	SOD (U/mg of protein)	LPO (n mol)
A	Normal control	10 ml/kg p.o	9.21±1.68	11.01±0.88	4.3±0.57
B	Toxicant control	Ethanol 3.76 mg/kg, p.o.	37.31±0.10	26.20±0.08	17.10±0.16
C	Standard	200 mg/kg, p.o+Ethanol	8.10±0.85**	6.34±0.10**	6.10±0.16**
D	MEAL	200 mg/kg, p.o+Ethanol	9.20±0.75**	8.11±0.42**	7.0±0.20**
E	MEAL	400 mg/kg, p.o+Ethanol	8.40±0.06**	7.13±0.20**	7.01±0.13**

Values are mean±SEM (n=6) one-way ANOVA, compared with the Group B; **$p<0.05$ is consider as statistical significant (n=6). MEAL: Methanolic extract of *Anogeissus latifolia*, SEM: Standard error of mean, CAT: Catalase, SOD: Superoxide dismutase, LPO: Lipid peroxidation

Table 7: Correlation study between metal ions with biochemical parameters

	Fe	Zn	Cu	SGPT	SGOT	ALP	TP	Ch	Tri
Fe	1								
Zn	0.426	1							
Cu	0.973**	0.207	1						
SGPT	0.999**	0.426	0.973*	1					
SGOT	0.920*	0.039	0.985*	0.920	1				
ALP	0.990**	0.295	0.995**	0.990**	0.966	1			
TP	0.999**	0.426	0.973*	0.999**	0.920	0.990*	1		
Ch	0.973**	0.207	0.999**	0.973*	0.985*	0.995**	0.973*	1	
Tri	0.999**	0.426	0.973*	0.999**	0.920	0.990**	0.999**	0.973*	1

Significant at **$p<0.05$. TP: Total protein, Ch: Cholesterol, Tri: Triglyceride, Fe: Iron, Cu: Copper, Zn: Zinc, SGPT: Serum glutamic pyruvic transaminase, SGOT: Serum glutamic oxaloacetic transaminase, ALP: Alkaline phosphatase
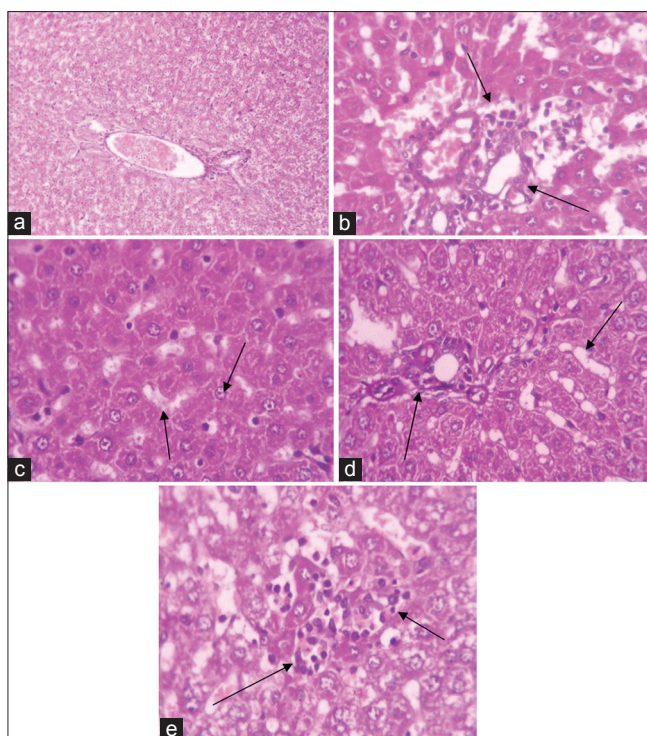
Fig. 1: Histopathology of the liver in ethanol-induced hepatotoxicity studies (×40 scale). (a) Normal control, (b) toxicant (ethanol), (c) standard (silymarin 200 mg/kg), (d) methanolic extract of *Anogeissus latifolia* (MEAL) (200 mg/kg), (e) MEAL (400 mg/kg)

Table 8: Correlation study between metal ions with oxidative agents

	Fe	Zn	Cu	CAT	SOD	LPO
Fe	1					
Zn	0.426	1				
Cu	0.973*	0.207	1			
Cat	0.999**	0.426	0.973	1		
SOD	0.973*	0.207	0.999**	0.973*	1	
LPO	−0.990	−0.295	−0.995**	−0.990	−0.995	1

Significant at **p<0.05. CAT: Catalase, SOD: Superoxide dismutases, LPO: Lipid peroxidation, Fe: Iron, Cu: Copper, Zn: Zinc

Table 9: Correlation study between metal ions with oxidative agents

	Cat	SOD	TP	Ch	Tri
Cat	1				
SOD	0.973*	1			
TP	0.999**	0.973*	1		
Ch	0.999**	0.973*	0.999**	1	
Tri	-0.999	-0.973	-0.999	-0.999	1

Significant at **p<0.05. CAT: Catalase, SOD: Superoxide dismutases, TP: Total protein, Ch: Cholesterol, Tri: Triglyceride

Table 9 indicated that CAT and SOD are positively correlated which are have significant reduction in TP, Ch, and Tri.

DISCUSSION

In the present study, the bark sample was collected in the month of August 2016, this is because of accumulation of plant secondary metabolites in bark is higher. Earlier literature are also revealed that after rainy season bark accumulates more secondary metabolites before winter that shows significant therapeutic activities [22,23]. Our investigation also showed the same trend when analyzed for

percentage yield. Further soil sample was analyzed for the presence of various metal ions as well as to know about the soil physicochemical nature. It was revealed that soil fertility and metal ions greatly play an important role for accumulation of secondary metabolites which further enhanced the yield and better therapeutic activities [24-26]. In the present study, content of Fe and Zn showed higher than other metals which are favorable for optimum growth and higher metabolic activities for plants. Earlier reports also suggested the same [27,28].

Yield of extract showed higher amount may be due to high content of Fe, Cu, and Zn and low content of non-essential heavy metals. Literature survey revealed that more extract procured from leaf samples when compared between three different geographical zones [25].

Liver participates in a variety of metabolic activities perhaps by virtue of the presence of number of enzymes and thus may self-expose too many toxicants, chemicals, and drugs which could injure it. In our hepatoprotective study, ethanol was used as hepatotoxicants to induce liver damage, since it is used by human beings for either medical or non-medical purposes. In this toxicity, there is increased formation of lipoperoxides, conjugated dienes, and malondialdehyde and reduced levels of antioxidants such as Vitamin E and glutathione in the tissues have been demonstrated in experimental animals administered with ethanol as well as alcoholic human subjects. The increased level of AST, ALT, and ALP are conventional indicator of liver injury [29]. Ethanol produces a constellation of dose-related deleterious effects in the liver [30]. In chronic alcoholics, hepatomegaly occurs due to accumulation of lipids and proteins in hepatocytes with an impaired protein secretion by hepatocytes [31,32]. Oxidative stress is one major factor in etiology of ethanol injury, mainly by Kupffer cells through the action of a substance called endotoxin, which is released by certain gram-negative bacteria present in the intestine, activates Kupffer cell to generates reactive oxygen species (ROS) and pro-inflammatory cytokines (tumor necrosis factor-alpha and interleukin-1), both of them can lead to liver damage [33]. In the experiment, MEAL extract at dose level of 400 mg/kg showed very significant results with respect to SGPT, SGOT, TP content, ALP, LPO Ch, and Tri and the values are nearby the standard value. Hepatotoxin gets converted into radicals in liver by action of enzymes and these attacks the unsaturated fatty acids of membranes in the presence of oxygen to give lipid peroxides consequently. The functional integrity of hepatic mitochondria is altered, leading to liver damage. During hepatic damage, cellular enzymes such as AST, ALT, and ALP present in the liver cells leak into the serum, resulting in increased concentrations [34]. Ethanol administration for 25 days significantly increased all these serum enzymes. Serum levels of SGPT can increase due to damage of the tissues producing acute hepatic necrosis, such as viral hepatitis and acute cholestasis. Alcoholic liver damage and cirrhosis also can associate with mild-to-moderate elevation of transaminases [35,36].

This is mainly due to the high content of accumulated plant constituents and metal ion contents especially Fe and Zn. Literature also evident that therapeutic activity is dependent on high accumulation of essential metals and also due to the presence of phenolic, flavonol, and flavonoid; it showed a significant result as antioxidant.

In the current study, treatment of rats with MEAL significantly (p<0.05 in 400 mg/kg b.w.) decreased the levels of SGPT in serum which is an indication of hepatoprotective activity.

SGOT is a mitochondrial enzyme released from heart, liver, skeletal muscle, and kidney. Liver toxicity elevated the SGOT levels in serum due to the damage to the tissues producing acute necrosis, such as severe viral hepatitis and acute cholestasis. Alcoholic liver damage and cirrhosis can also associate with mild-to-moderate elevation of transaminases. In the current study, treatment of animals with MEAL bark significantly (p<0.05) decreased the levels of SGOT in serum which is an indicative of hepatoprotective activity.

In case of toxic liver, alkaline phosphatase levels are very high, which may be due to defective hepatic excretion or by increased production of ALP by hepatic parenchymal or duct cells 95. In the current study, treatment of animals with MEAL bark significantly (p<0.05 in 400 mg/kg b.w) decreased the levels of ALP in serum as an indication of hepatoprotective activity.

Toxicant induces hypercholesteremia and hypertriglyceridemia, may be due to the activation of enzyme HMG CoA reductase, the rate-limiting step in Ch biosynthesis. The increased serum Tri level in ethanol-treated rats may be due to the decreased activity of lipoprotein lipase, which is involved in the uptake of Tri -rich lipoprotein by the extrahepatic tissues [37]. Pre-treatment with MEAL bark reduced the elevated Ch and Tri levels, suggesting that the extracts prevented ethanol-induced hyperlipidemia probably due to their hepatoprotective activity. Treatment with MEAL significantly (p<0.05 with 400 mg/kg b.w) reduced the levels of Ch and Tri in ethanol-induced hepatotoxic animals, indicating the hepatoprotection.

Liver toxicity decreases the TP level in serum due to the damage to the tissues. Since the MEAL bark show increase in TP level in serum of animals, it possesses statistically significant (at p<0.05) hepatoprotective activity.

Our study further revealed that chronic exposure to ethanol decreased the activities of the ROS scavenging enzymes, namely, SOD and CAT. This is in line with assumption suggested earlier that decrease in the activity of antioxidant enzymes SOD and CAT following ethanol exposure may be due to the damaging effects of free radicals, or alternatively could be due to a direct effect of acetaldehyde, formed from oxidation of ethanol, on these enzymes [38]. In our studies, it reveals that MEAL could restore the activity of both these antioxidant enzymes and possibly could reduce the generation of free radicals and hepatocellular damage.

Formation of ROS, oxidative stress, and hepatocellular injury has been implicated to alcoholic liver disease. It has been documented that Kupffer cells are the major sources of ROS during chronic ethanol consumption, and these are primed and activated for enhanced formation of pro-inflammatory factors. In addition, alcohol-induced liver injury has been associated with increased amount of LPO. Indeed, MEAL supplementation in our study was potentially effective in blunting LPO, suggesting that MEAL possibly has antioxidant property to reduce ethanol-induced membrane LPO.

Although various enzymatic and non-enzymatic systems have been developed by cell to cope up with the ROS and other free radicals, when a condition of oxidative stress establishes, the defense capacities against ROS becomes insufficient [39]. ROS also affects the antioxidant defense mechanisms, reduces the intracellular concentration of GSH, and decreases the activity of SOD and CAT. It has also known to decrease the detoxification system produced by glutathione-S-transferases. Increasing evidence indicates that oxidative stress causes liver injury, cirrhosis development, and carcinogens. In our studies, it reveals that MPME could restore the activity of both these antioxidant enzymes.

Correlation coefficient study revealed the positive impact on biochemical estimations as well as procurement of extracts and antioxidant activity of the MEAL bark extracts. It indicated that high metal ions accumulation have positive correlation on accumulation of secondary metabolites which further plays an important role in therapeutic activity, that is, hepatoprotective activity. Earlier reports are also revealed the same [40,41].

CONCLUSION

The present study established the powerful hepatoprotective activity with the selected dose at 200 and 400 mg/kg b.w, respectively, with MEAL bark extract and resulted dose-dependent activity. The activity was depend on the collection from geographical location as well as time of collection of the plant parts. Further soil nature also revealed

the important action which is neglected by many of the research article that indicated significant correlation for the better hepatoprotective activity of the selected plant sources. It is positively correlated that any therapeutic activity is also correlated with the essential metal ion content as well as their impact on procurement of extracts and other parameters. Hence, finally, the results concluded that soil nature, cultural zone, and time of harvest play an important role in accumulation of plant secondary metabolites as well as better therapeutic activity.

AUTHOR'S CONTRIBUTION

Prateek Bisht: He is the PhD scholar. The whole research work of this manuscript is carried out by him. Analysis and interpretation of results are carried out by him. Chandrashekhara S: He is supervisor in this PhD study. The whole research work designed by him as well study concept. Further acquisition of data carried out by him. Kuntal Das: Critical revision of the drafted manuscript is carried out by him. He framed and designed the manuscript as per the journal format. Finally, all authors are approved the manuscript for publication. Sourav Tribedi: He has drafted the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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