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Research Article

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Hepatoprotective Effect of Ethanolic Extracts of *Allophylus Cobbe* (L.) Raeusch leaves against Paracetamol Induced Hepatotoxicity in Albino Wistar Rats

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Abstract: Liver disorders are one of the major concerns globally. Various conventional therapeutics used in the treatment of liver diseases are sometimes not enough and associated with serious side effects. Therefore, herbal medicines could be promising to defeat the above problems, and to treat diseases effectively. Aim of the current investigation is to screen the hepatoprotective activity of the ethanolic extract of *Allophylus cobbe* (EEAC) leaves against Paracetamol (PCM) induced hepatotoxicity in rats. EEAC (200 and 400 mg/kg) were administered to the rats for 7 days and hepatotoxicity was induced by administration of PCM (3 g/kg) on the 8th day. After 24 h of toxicity induction, the blood samples were collected and serum and tissue biochemical parameters like- serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase(ALP), acid phosphatase (ACP), Creatinine, superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), lipid peroxidation (LPO) and total proteins were analyzed. Livers of the animals were isolated and were studied for histopathological changes. The extract treated animals were compared with the animals treated with standard hepatoprotective Silymarin (100 mg/kg). Administration of PCM causes hepatotoxicity to the animals, EEAC and silymarin prevented the PCM induced hepatotoxicity. The level of the increased blood biochemical parameters were significantly decreased by oral administration of EEAC and silymarin. PCM hepatotoxicity raised the LPO activity of the liver tissue which was significantly decreased by EEAC and silymarin. Decrease in protective tissue enzymes (SOD, CAT and GSH) and the proteins by the PCM hepatotoxicity were significantly increased by the EEAC and silymarin. Histopathological observation of the PCM treated group showed the marked degeneration of the liver cells and liver damage which was significantly restored when the animals were treated with the EEAC and silymarin. *Allophylus cobbe* showed the presence of bioactive components in the plant having the antioxidant potential; which might be responsible for hepatoprotective activity of the plant. The present study showed that EEAC restored the levels of altered biochemical parameters and prevented the liver from the toxic effects of PCM revealing the hepatoprotective potential of *Allophyluscobbe*.

Keywords: Hepatoprotective activity, Silymarin, Paracetamol, Allophylus cobbe, hepatotoxicity.

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1. INTRODUCTION

The liver is one of the important organs of human and other living organisms. The functional integrity of liver can be maintained by certain kinds of biochemical functions and metabolic activity. Human liver is known for different major metabolic functions for example, metabolism of bilirubin, porphyrin, bile acids, amino acids, proteins, carbohydrates, lipids, hormones, vitamins, biotransformation and detoxification functions, alcohol degradation, acid- base balance and so on. Thus liver is the important organ required to maintain the body's homeostasis.[1] Worldwide, over the last several years, liver diseases have increased enormously to become one of the leading causes of death and illness. According to the global burden of liver disease, Liver disease accounts for approximately 2 million deaths per year worldwide, which are due to cirrhosis, viral hepatitis and hepatocellular carcinoma. Recently cirrhosis is the 11th most common cause of death and liver cancer is the 16th leading cause of death; collectively, they account for 3.5% of all deaths globally.[2] Acetaminophen (PCM) is the most commonly used analgesic worldwide and recommended as first-line treatment in all pain conditions by WHO.[3] It is also used for its antipyretic effects, helping to reduce fever.[4] PCM induced hepatotoxicity is well established experimental model to determine the hepatoprotective activity of new pharmacological agents.[5] Therapeutic dose of PCM is considered safe for therapy but at higher doses, PCM can cause centrilobular necrosis that eventually leads to liver failure.[6] The major advantage of PCM model is that it is a clinically relevant model and is a dose dependent hepatotoxicant.[7] At therapeutic doses, PCM is metabolized by glucoronidation or sulfation by cytochrome P_{450} system and the excess of it get converted into the reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI),[8] Under normal condition, NAPQI is bio-converted into non-toxic metabolites by the enzyme glutathione (GSH). However, in case of overdose, excess NAPQI depletes GSH content and binds covalently to hepatic cellular proteins resulting in mitochondrial dysfunction and mitochondrial oxidative stress that eventually induces necrosis and apoptosis of hepatocytes.[9] Thus PCM-induced hepatotoxicity has been studied for several years due to their detrimental effects on health. *Allophylus cobbe* is a small shrub tree from the family Sapindaceae commonly known as Tippani in Marathi grows up to 5 m. Allophylus cobbe has strong ethnobotanical and ethno-pharmacological background. The bark is bitter, sweet and astringent. It has digestive, carminative, constipating and anti-inflammatory properties. It is useful in ulcers, wounds, dyspepsia, anorexia, diarrhea, stomachache, fever, bruises and inflammation. In Konkan region the bark is used in bone fractures and dislocation of joints. The leaf extract is taken against stomachache and leaf pest is applied on scabies. The root power mixed with honey is a remedy for diarrhea whereas leaf juice effectively combats the ulcers. The fruits are cooling, sweet and tonic and are advised in general debility.[10] Amid these enormous medicinal values, the current investigation is aimed to investigate the hepatoprotective activity of *Allophylus cobbe* in PCM-induced hepatotoxicity rat model.

2. MATERIALS AND METHODS

2.1. Plant material collection Preparation of plant extract

The leaves of the *Allophylus cobbe* were collected from the forest of Jyotiba, Kolhapur (District), Maharashtra, India. Leaves were identified and authenticated by the Botanical Survey of India, Pune with specimen voucher number-BSI/WRC/IDEN.CER./2018/H3-69-SDC 02 of the plant deposited at the same. The leaves were shade dried for a week and finely powdered using the blender. The powder was passed through the 100 mesh sieve size and stored in sealed polythene bags. The powered drug (100 g) was extracted with 500 ml ethanol for more than 6 hr by using the soxhlet extraction assembly and concentrated by rotary evaporation and vacuum drying. The yield of plant was recorded (4.5 %) and stored at -20°C until further use.

2.2. Chemicals

PCM and Silymarin were obtained from E Merck (India) Ltd. Mumbai and Ranbaxy Laboratories Ltd. Baddi, H.P., respectively. SGPT, ALP, ACP, and Creatinine were estimated by using diagnostic kit obtained from Pathozyme Diagnostic Ltd., Kagal, Kolhapur and Medsource Ozone Biomedicals Pvt. Ltd. All reagents were of analytical grade.

2.3. Maintenance of animals and their feeding

Albino Wistar rats of either sex (150-180 g) were obtained from Crystal Biological Solutions, Uruli Devachi, Pune Maharashtra. Rats were housed in standard polypropylene cages (3 animals per cage) and were maintained under standard hygienic conditions at 25-28^0 C with 12 hr light/dark cycle and provided with standard pellet diet procured from Pranav Agro, Sangli. Water and food was given *ad libitum* throughout the study. The animals were cared for and maintained as per the approved guidelines of the "Committee for the Purpose of Control and Supervision of Experiments on Animals" (CPCSEA, India) and the protocol was approved by the Institutional animal Ethical Committee, Rajarambapu college of Pharmacy, Kasegaon (1290/PO/Re/S/09/CPCSEA, Protocol No. RCP/P-18/18-19 Dated: 16/03/2019).

2.4. Preparation of doses and treatments

The PCM was given to animals at a dose of 3 g/kg orally. Aqueous suspension of EEAC was prepared in distilled water and different doses of extract (200 and 400 mg/kg) and silymarin (100 mg/kg) were given to animals orally.

2.5. Acute oral toxicity

The acute oral toxicity was performed as per the OECD set guidelines, revised draft guidelines 423 method[11]. Female non-pregnant rats were used for the study. Animals were weighed and test substance was given in a single dose orally through rat oral needle. After the administration of test substance, food was withheld for 2 hrs, but not water. Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hrs, with special attention given during the first 4 hrs, and daily thereafter, for a total of 14 days (OECD, 2001).[11]

2.6. Evaluation of Hepatoprotective activity

Treatment groups: Albino wistar rats of either sex weighing 150-180 g were randomly divided into 05 groups of six animals each for the determination of pharmacological activity by biochemical and histopathological parameters.

Group 1- served as negative control and animals were given distilled water for 7 days.
Group 2- served as positive control to which hepatotoxicity was induced by using PCM. The animals were given distilled water for 7 days and given PCM single dose (3 g/kg body weight) [12] orally on day 8
Group 3- was given the standard drug Silymarin at a dose of 100 mg/kg p.o.[13] for 7 days (p.o.) followed by a single dose of PCM on day 8.
Groups 4-5 were treated with 200 and 400 mg/kg of ethanol extract of Allophylus cobbe for 7 days (p.o) followed by a single dose of PCM on day 8.

After the study period, the animals were sacrificed using ether anesthesia. Blood samples were collected by the retro orbital plexus and the serum was separated for evaluating biochemical parameters. The liver was immediately removed and a small fraction was homogenized for tissue biochemical assay and small pieces were fixed in 10% formalin for histopathological assessment. [14]

2.7. Blood biochemical assay

The level of different hepato specific marker enzymes were estimated by reported methods *viz*, SGOT and SGPT by Modified Reitman & Frankel's method, [15] ALP by Modified Kind & King's method, [16] ACP by Modified King's Method [17] and creatinine by Modified Jaff's Method. [18] Enzyme activities were measured by using the diagnostic strips and were read on colorimeter.

2.8. Tissue biochemical assay

Lipid peroxidation was determined by measuring Malondialdehyde (MDA) by Okhawa et al. method[19] Glutathione was measured by its reaction with 5, 5'-dithiobis (2-nitrobenzoic acid) DTNB by Ellman's method [20] Catalase by Aebi et al. 1974 [21] method and Superoxide dismutase was determined by Marklund et al. 1982 [22] method and the total protein content was measured by Lowry et al. method.[23]

2.9. Histopathological observation

The livers from the different groups were isolated and grossly examined for any pathological changes and then fixed in 10% formalin, dehydrated in graduated ethanol (50%-100%), cleared in xylene and embedded in paraffin. 5µm thick sections were prepared, stained with haematoxylin and eosin dye and microscopically examined for histopathological changes.

2.10 Statistical Analysis

The data are expressed as mean ± standard error of mean (mean ± SEM). The difference among means has been analyzed by one way ANOVA. The results of all the extracts including standard drugs are compared with the result produced by the positive control group. A value of $p< 0.05$ was considered as statistically significant.

3. RESULT

3.1. Phytochemical screening

The presence of poly phenolic compounds, tannins, flavonoids, alkaloids and Saponins were observed in ethanolic extract. *[15-18]*

3.2. Acute toxicity study

Acute toxicity study exhibited safety of ethanolic extract at a dose of 2000 mg/kg body weight. All animals were alive, active and healthy during the observation period. Hence 1/10th and 1/5th dose (200 mg/kg and 400 mg/kg) of the drug was selected for the activity. *[15-18]*

3.3. Blood biochemical estimation

The detailed results of blood biochemical estimation are presented in Table 1. Administration of PCM induced significant increase in the enzymatic activities of SGOT, SGPT, ACP, ALP and Creatinine ($P < 0.05$) as compared to normal control group. Oral administration of extract at different doses (200 mg/kg and 400 mg/kg) and silymarin (100 mg/kg) significantly ($P < 0.05$, $P < 0.01$, $P < 0.001$) decreased elevated levels of serum enzymes to its normal compared to PCM treated rats in both preventive and curative studies.*[15-18]*

Table 1: Effect of EEAC on blood biochemical parameters in control and experimental groups of animals

Treatment	SGOT Activity in IU/L	SGPT Activity in IU/L	ACP KA units	ALP KA units	Creatinine mg/dl
Normal control	90.7±14.111	43.3±8.819	1.63±0.2522	4.89±0.3933	1.20±0.2309
Paracetamol 3 g/kg	405.3±66.826$^{###}$	293.3±17.638$^{#####}$	7.25±1.299$^{####}$	15.29±0.3406$^{#####}$	5.07±0.8110$^{###}$
Standard (Silymarin) 100 mg/kg	165.3±37.333**	66.7±20.276***	2.41±0.2205***	5.09±0.7091****	1.6±0.2309***
Allophyluscobbe 200 mg/kg	250.7±46.495ns	166.3±55.167*	5.33±0.0833ns	11.17±1.225**	2.4±0.2309**
Allophyluscobbe 400 mg/kg	213.3±28.221*	130.00±11.547**	4.00±0.3819*	7.64±0.6784***	1.87±0.4807***

All the values are expressed in mean ± SEM (n=06), where, ns- indicates p > 0.05, *p < 0.05, **p < 0.01 and ***p < 0.001 was considered as statistically significant when compared with the PCM treated group and #p < 0.05, ## p < 0.05 and ###p < 0.001 was considered as statistically significant when compared with the normal control group.

3.4. *Tissue biochemical estimations*

Different biochemical estimations were performed in liver tissues which are presented in Table 2. A significant increase was observed in the level of LPO in the liver when compared with control group ($P < 0.05$). Treatment with different doses of the extract and the standard drug silymarin inhibited the LPO in dose dependant manner and reversed the oxidative stress towards the normal control ($P < 0.05$). GSH, CAT and SOD are supposed to be an important endogenous defense against peroxidative destruction of cellular membrane. In the present study administration of PCM significantly decreased the levels of GSH, SOD and CAT ($P < 0.05$). Treatment with the different doses of extract and standard silymarin effectively restored the levels of GSH, SOD and CAT ($P < 0.05$) towards the normal control (Table 2). All two doses of extract improved GSH, SOD and CAT levels in the liver; however, therapy at 400 mg/kg was more effective. Total protein level was significantly decreased by PCM intoxicated animals that were restored towards normal by the doses of extract and standard silymarin significantly; however, maximum restoration was observed at 400 mg/kg ($P < 0.05$).

Table 2: Effect of EEAC on tissue biochemical estimations in control and experimental group of animals.

Treatment	SOD U/min/ g protein	CAT U/mg of protein	GSH nmol/ min / mg protein	LPO moles MDA/ mg proteins/ ml	Total proteins mcg/ml
Normal control	285.71±7.140	4.372±0.0496	0.4632±0.0347	30.96±3.491	188.74±7.140
Paracetamol 3 g/kg	64.28±12.373$^{\#\#\#}$	1.319±0.3284$^{\#\#\#\#}$	0.1298±0.0160$^{\#\#\#\#}$	178.05±9.820$^{\#\#\#\#}$	32.76±8.152$^{\#\#\#}$
Standard (Silymarin) 100 mg/kg	242.85±7.143**	3.586±0.0797****	0.3357±0.0301***	81.28±23.041**	167.15±9.350***
Allophyluscobbe 200 mg/kg	157.13±68.140 ns	2.139±0.0986*	0.2622±0.0171*	116.12±10.991*	80.79±6.811ns
Allophyluscobbe 400 mg/kg	221.42±7.143 *	2.618±0.2079***	0.2989±0.0106**	97.73±5.121**	142.86±16.853**

All the values are expressed in mean ± SEM (n=06), where, ns- indicates p > 0.05, *p < 0.05, **p < 0.01 and ***p < 0.001 was considered as statistically significant when compared with the PCM treated group and #p < 0.05, ## p < 0.05 and ###p < 0.001 was considered as statistically significant when compared with the normal control group.

3.5. *Histopathological observation*

Liver sections of the normal animal showed normal hepatic cells with conserved cytoplasm, prominent nucleus and nucleolus and central vein (Fig 1a). Severe degree of liver damage, congestion and macro and micro- vesicular steatosis was observed in liver sections PCM treated animal (Fig 1b). In silymarin (100 mg/kg) treated animals liver section showed mild degree of liver damage, sinusoidal congestion, mild inflammation and mild degree of macro and micro- vesicular steatosis (Fig 1c). Animals treated with EEAC (200 and 400 mg/kg) due to restoration of the hepatocytes liver section showed mild degree of liver damage, sinusoidal congestion, mild inflammation and mild degree of macro and micro-vesicular steatosis (Fig 1d, 1e). Hence these results indicated a Hepatoprotective potential of the extract.

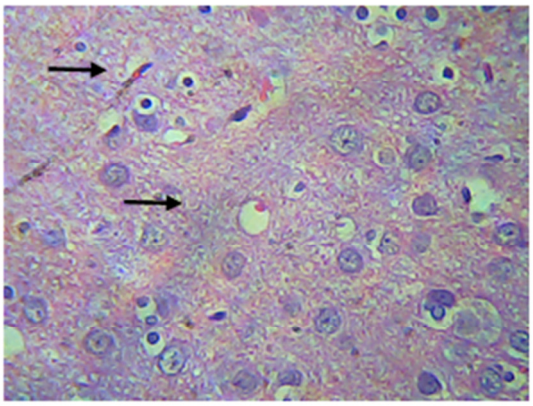

Fig 1A. Normal control rat liver

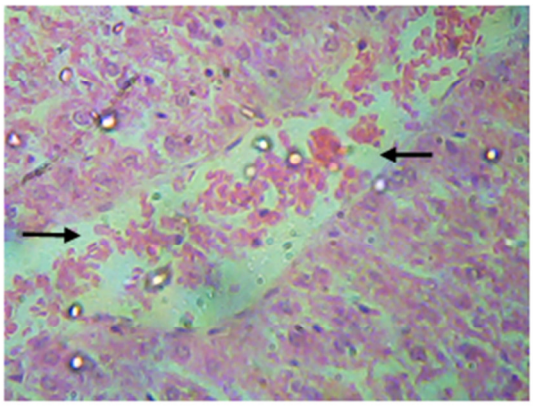

Fig 1B. PCM Induced rat liver

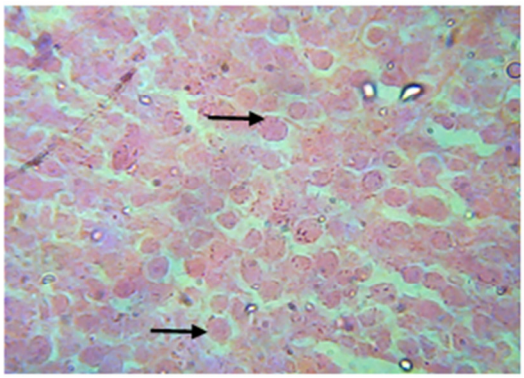

Fig 1C. PCM +Standard Silymarin (100 mg/kg) treated rat liver

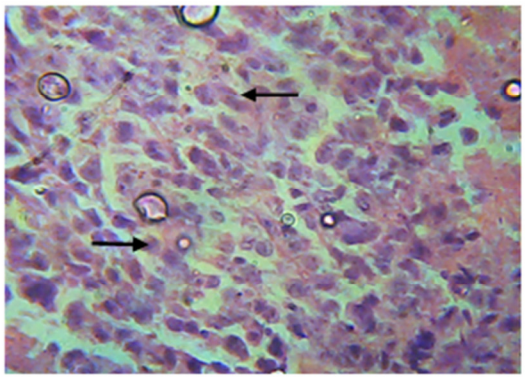

Fig 1D. PCM + EEAC (200 mg/kg) treated rat liver

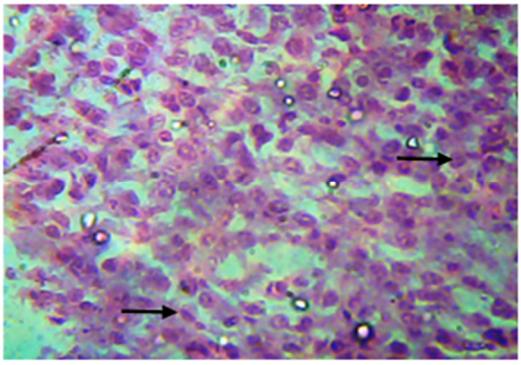

Fig 1E. PCM + EEAC (400 mg/kg) treated rat liver

Fig. 1 A-E: histopathology of liver sections in normal and experimental groups of rats under 40X magnification. Fig. 1 A: Normal control group- with well defined liver architecture with normal hepatic cells with conserved cytoplasm. Fig. 1 B: PCM Treated group- showed the degenerative hepatocytes with necrosis causing severe degree of liver damage. Fig. 1 C: standard silymarin (100 mg/kg) treatment group- showed the mild degree of liver damage and restoring hepatocytes. Fig. 1 D and E: group treated with Allophylus cobbe (200 mg/kg and 400 mg/kg) showed the regeneration of liver cells with mild degree of liver damage.

4. DISCUSSION

PCM at therapeutic doses is most preferred analgesic and antipyretic substance, but when the therapeutic dose exceeds it can produce hepatic necrosis in experimental animals and humans. Alteration in permeability of the cells affects the transport function of hepatocytes causing the leakage of cellular enzymes into the plasma and is the main sign of the PCM induced hepatic injury. Majority of the metabolic reactions are carried out by the liver therefore it is an essential organ which is affected by various chemicals and toxins and liver injuries by various hepatotoxins are main toxicological problems at present.[24] Herbs catch the attention for treatment of various liver diseases to recur the lack of reliable liver protective. Phytochemical investigation of ethanolic extract of *Allophylus cobbe* showed the presence of poly phenolic compounds, tannins, flavonoids, glycosides, alkaloids and saponins. Previous Phytochemical studies with *Allophylus cobbe* showed the presence of several terpenoids, saponins[25] seven compound mixture of terpenoids, alkanes and fatty acids, 1,1-diethoxy ethane, phytol and hexanoic acid. *Allophylus cobbe* has wide Ethno-medicinal importance and is used in different biological activities like Antiosteoporotic, Wound healing, anti-inflammatory, Antiulcerogenic, ulcer healing activity, Antidiabetic, Antihypertensive, Antibacterial, Antimalarial, Insecticidal [10] hence it proves its medicinal importance. It is well known that PCM administration alters the hepatic cell membrane permeability, [26] that causes the introduction of cytochrome or depletion of hepatic glutathione [27] and thereby leakage of several enzymes (SGOT, SGPT, ACP, ALP and creatinine) in serum. Reduction in levels of these enzymes is one of the several mechanisms to combat the hepatotoxicity. Notably, ethanolic extract of *Allophylus cobbe* reduced leakage of enzymes in serum, signifying that they protect the liver cell and also maintained normal liver functioning and further stabilized plasma membrane as well as regeneration of damaged liver cells by restoring the hepatic parenchymal cells and normalizing the altered cell permeability. Antioxidant protective enzymes such as SOD,CAT and GSH are vital in protecting organisms from oxidative damages. SOD converts superoxide radicals to hydrogen peroxide while CAT present in peroxisomes of eukaryotic cells converts hydrogen peroxide to water and oxygen.[28] GSH is the main intracellular nonprotein sulfhydryl containing compound and one of the essential endogenous antioxidants necessary to maintain cellular proteins and lipids in their functional states. Hepatotoxicity characterized by conjugation of excess of NAPQI and GSH diminish the GSH stores and results in formation of GSSG (Glutathione oxidized). This further induces the toxic effects of oxidative stress and remarkably damage the membrane and cells.[29] In the present study, ethanolic extract of *Allophylus cobbe* showed an increase in the concentration of GSH which maintained the cellular proteins and lipids in their functional states; it also increases the levels such as SOD and CAT whose concentration was decreased by the liver injury induced by PCM. Lipid peroxidation is induced by oxidative stress on cell-membrane lipids. Toxic products of lipid peroxidation cause extensive damage of macromolecules. Product of lipid peroxidation Malondialdehyde (MDA) determination is commonly used to evaluate lipid peroxidation in animal tissues.[30] Ethanolic extract of *Allophylus cobbe* decreased the lipid peroxidation in PCM treated animals and is concluded

by decrease in MDA levels. The mechanism of hepatoprotection by the Ethanolic extract of *Allophylus cobbe* leaves might be due to its antioxidant potentials, it concludes that the extract reduces the ROS that may decrease the oxidative stress on liver cells and increases the functioning of liver antioxidant enzymes thus prevent the liver from the toxic effects of PCM. Regeneration of the hepatocytes by the improved synthesis of protein can also be the probable mechanism for the hepatoprotective activity. The above study revealed that PCM induced liver damage in albino wistar rats, was significantly decreased after being treated with the ethanolic extract of *Allophylus cobbe* leaves, which revealed its hepatoprotective activity.

5. CONCLUSION

In the present study, hepatotoxicity is induced in the rats using PCM. The hepatoprotective activity in rats displayed that EEAC (200 and 400 mg/kg) can significantly protect the liver from the damaging effects of PCM in dose dependent manner. Besides, EEAC showed decreased serum levels of liver enzyme markers. Thus, EEAC was found to be effective in the protection of liver. However, there is need to ascertain the actual active constituents responsible for hepatoprotective activity, and underlying hepatoprotective mechanism of EEAC. In this way, herbal medicines could be a potential approach for the effective treatment of liver ailments with minimum side effects.

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7. AUTHORS CONTRIBUTION STATEMENT

The submitted research work is guided by Dr. Remeth Dias and Dr. Chandrakant Magdum and laboratory research work; experiments were carried out by Mr. Sandeep Chavan.

8. CONFLICT OF INTEREST

Conflict of interest declared none.

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