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Hepatoprotective Effect Of Clerodendrum Serratum Seeds Extract On Paracetamol Induced Hepatotoxicity

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Abstract: *Hepatic damage is associated with distortion of its metabolic functions and it is still a major health problem.Unfortunately many synthetic drugs used in the treatment of liver diseases are inadequate and also cause serious side effects. Clerodendrum serratum (Verbenaceae) is an important medicinal plant growing in the tropical and warm temperate regions like Africa, Southern Asia; Malaysia and distributed throughout in forests of India and Sri Lanka. It is traditionally valued and reported for treating pain, inflammation, rheumatism, respiratory disorders, fever and malarial fever in India with a long history. The aim of this study was to evaluate the hepatoprotective effects of ethayl acetate and ethanol extract of Clerodendrum serratum seed. The values of AST, ALT, ALP, and total bilirubin were all substantially raised by paracetamol. Significant hepatoprotective effects were obtained by pretreatment with Clerodendrum serratumextract. Results of the ethanolic extract of Clerodendrum serratumwere comparable to Silymarin.The current investigation supports the hepatoprotective properties of Clerodendrum serratumseed extract.*

Keywords:*Clerodendrum serratum extract, hepatoprotective, paracetamol.*

INTRODUCTION: The liver is of vital importance in intermediary metabolism and in detoxification and elimination of toxic substances. The liver is often affected by a multitude of environmental pollutants and drugs, all of which place a burden on this vital organ and can damage and weaken it, eventually leading to diseases like hepatitis or cirrhosis [1]. Paracetamol's hepatotoxicity is caused by its reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI), which causes oxidative stress and glutathione (GSH) depletion. Paracetamol toxicity is due to the formation of toxic metabolites when a part of it is metabolized by cytochrome P450 [2]. Introduction of cytochrome or depletion of hepatic glutathione is a prerequisite for paracetamol-induced hepatotoxicity. In spite of tremendous strides in modern medicine, the treatment of liver disorders isinadequate and many formulations containing herbal extracts are used for regeneration of hepatic cells and for protection of the liver against damage [3].

Hepatotoxicity or liver damage is characterized by metabolic dysfunction and histopathological patterns such as neoplasms, vascular lesions, granuloma, steatosis, cholestasis, hepatitis, and zonal necrosis, which represents 5% of all injuries, making it frequent to known injury. As a consequence, there are more than a million casualties reported in a year, which are indirectly due to liver disfigurement or hepatocellular carcinoma. Conventional or synthetic drugs protect us from various external and internal ailments. Unfortunately, the drugs used to treat liver diseases are dis-satisfactory because they do not offer complete protection to the organ and can exert serious long-term side effects [4, 5]. Hepatic damage is associated with distortion of its metabolic functions and it is still a major health problem. Unfortunately many synthetic drugs used in the treatment of liver diseases are inadequate and also cause serious side effects [6]. In view of severe undesirable side effects of synthetic agents, there is growing interest in evaluating traditional herbal medicines that are claimed to possess hepatoprotective activity. A single drug cannot be effective for all types of severe liver diseases. Therefore, an effective formulation using indigenous medicinal plants has to be developed with proper pharmacological experiments and clinical trials [7]. Therefore, it is crucial to understand the role of plants as an alternative to cure liver damage. Plants are understood to have satisfactory clinical efficacy with low adverse side effects.

Clerodendrum serratum (Verbenaceae) is an important medicinal plant growing in the tropical and warm temperate regions like Africa, Southern Asia; Malaysia and distributed throughout in forests of India and Sri Lanka. It is traditionally valued and reported for treating pain, inflammation, rheumatism, respiratory disorders, fever and malarial fever in India with a long history.C. serratum (Bharangi) is widely used in indigenous systems of medicine for the treatment of respiratory disease, especially asthma, and several other diseases [8-9].

Ayurvedic science has propagated the use of C. serratum as an effective herb in treatment for asthma, body ache, cholera, eye disorder, ulcers, snakebite, wound, tuberculosis, and cancer. Root and leaves of C. serratum have been explored for various activities, but seeds of this plant have not been explored for hepatoprotective activities. Therefore, the present research work was aimed to screening of hepatoprotective activities of the plant seeds and establish scientific basis for the same. However, to the best of our knowledge, there is no proper scientific evidence available on the hepatoprotective activity of theC. serratum seed. [10-11]. Therefore, the aim of this study was to investigate the hepatoprotective activity of the ethanolic extract of C. serratum seedin paracetamol induced hepatic damage in rats.

Animals and care: Healthy albino rats of either sex, weighing between 180-250g disease free animal were used. They were housed in standard environmental conditions of temperature, humidity, and light and provided with standard rodent food and water ad libitum as per IEAC guideline (IEAC/918/CPCSEA/5 date 29.4.2022).

Procurement of Clerodendrum Serratum seed: Clerodendrum Serratum seed were procured from local market of Bhopal and authenticated by botanist. The seeds were pulverized into small pieces, dried in sun and ground with the help of an electrical grinder to get powder, stored in airtight containers and used for phytochemical and pharmacological studies.

Preparation of extract: Clerodendrum Serratum seedpowdered (100 g) was successively extracted with the following solvents of increasing polarity in a soxhlet apparatus.The dried powder was packed in Soxhlet apparatus extracted with petroleum ether. After the extraction, extract filtered and solvent was removed with the help of rotatory evaporator. The same process was carried out to get ethyl acetate and ethanol extracts. The total yield of the extracts obtained after removing the solvents was calculated. All the extracts were concentrated by distilling the solvents and the extracts were dried in an oven. Each time before extracting with the next solvent, the marc was dried in an air. The extracts were then subjected to various qualitative test using as per following to determine the presence of various phytoconstituents such as alkaloids, glycosides, flavonoids, carbohydrates, aminoacids, saponins, sterols and terpenoids, cardiac glycosides, coumarins, carotenoids, tannins, phenolic compounds, fixed oils and fats etc [12].

Acute toxicity study: Acute toxicity studies of extracts of Clerodendrum Serratum seeds were performed in Swiss Albino rats dose levels of 50, 300 and 2000 mg/kg as per OECD guide lines. No mortality was observed in animals dosed with the extracts of Clerodendrum Serratum seeds at dose levels of 50, 300 and 2000 mg/kg (p.o). The treated animals did not demonstrate any significant changes in behavioral pattern and exhibited normal activity.

Preparation of extract: dried extracts were redissolved in water using carboxymethyl cellulose (CMC) as suspending agent and this suspension was used for hepatoprotectiveand gastro-protective activity.

Hepatoprotective activity

Experimental design and drug administration: Thirty wistar albino rats were randomly divided into seven groups containing six rats each. The treatment of each group is described below. Paracetamol 1000 mg/kg p.o.for 7 days administered orally once on the first day.

Table 1: Study design for hepatoprotective effect of Clerodendrum Serratumextracts

Normalcontrol	Normal saline (0.85%)
Disease control	Paracetamol 1000 mg/kg p.o.for 7 days
Standard	(silymarin treated) + Paracetamol (1000 mg/kg p.o.)
EACS (100 mg/kg)	Ethyl acetate extract of Clerodendrum Serratum(100 mg/kg)
EACS (200 mg/kg)	Ethyl acetate extract of Clerodendrum Serratum(200 mg/kg)
EOCS (100 mg/kg)	Ethanol extract of Clerodendrum Serratum(100 mg/kg)
EOCS (100 mg/kg)	Ethanol extract of Clerodendrum Serratum(200 mg/kg)

Mortality: During the whole length of the trial, morbidity and mortality were monitored twice daily (in the morning and in the evening) for each of the animals. This was done throughout the acclimatization process.

Body weight: The weight of the body was measured on day 0 (before to the administration of the dose) and day 8 of the study. On day 8, the percentage change in body weight was determined by using the prior body weight as the basis.

Serum biochemical analysis: Liver biochemical tests (AST, ALT, and ALP) were performed on a clinical chemistry automatic analyzer (ADVIA 2400, Bayer Diagnostics). AST, ALT, and ALP were measured according to the previous methods using commercial assay kit (Bayer Diagnostics). The serum total concentrations of bilirubin were measured adhering to the scientific methods utilizing commercial kits on the Express Plus biochemical analyzer (Ciba-Corning Diagnostics) [13].

Assessment of liver function parameters: At the end of the experimental period, animals were sacrificed by cervical decapitation under mild ketamine anesthesia, blood was collected and the serum was separated by centrifuging at 300 rpm for 10 min. The collected serum was used for the assay of marker enzymes. The serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) were estimated [14, 15]

ASSESSMENT OF IN-VIVO LIVER ENZYMATIC ASSAYS

Superoxide dismutase (SOD) assay: A volume of 880 µl of 0.05 M carbonate buffer (pH 10.2) containing 0.1 mmol EDTA and 20 µl of 30 mmol epinephrine in 0.05% acetic acid was added to the tissue extract of 100 µl, and changes in activity were measured at 480 nm for 4 min. The activity was expressed as the amount of enzyme that inhibits the oxidation of epinephrine by 50%, which is equivalent to one unit and is expressed in terms of units/mg protein [16].

Catalase (CAT) assay: The reaction solution of catalase activity contained 1 ml of 59 mmol/L H2O2 (dissolved in 50 mmol phosphate buffer, pH 7.0) and 0.1 ml of hepatic supernatant was added to 1.9 ml deionized water. Changes in the absorbance of the reaction solution at 240 nm were determined every 1 min up to 3 min (using Kinetics spectrometer). One unit of catalase activity was defined as an absorbance change of 0.01 as units/mg/min [17].

Malondialdehyde (MDA) content: Malondialdehyde (MDA), a marker of lipid peroxidation, was assessed by the method of, using 1, 1, 3, 3-tetramethoxypropane as standard. Briefly, 8.1% SDS was added to the tissue homogenate and incubated for 10 min at RT, followed by boiling with 20% acetic acid and 0.6% thiobarbituric acid (TBA) for 1 h in a water bath. After cooling, butanol: pyridine solution (15:1 v/v) was added and the mixture was centrifuged at 600 × g for 5 min. The absorbance of the upper coloured layer was measured at 532 nm and the concentration of MDA was expressed in terms of nmol/mg protein [18].

Histological studies: The stomachs were immersed in 10% formalin solution for histopathological examination. These tissues were processed and embedded in paraffin wax. The central part of damaged or ulcerated tissue was cut on half along the long diameter. If the stomach was protected from the damage then the section was taken from basal part using a rotary microtome sections of thickness of about 5µm were cut and stained with Haemotoxylin and Eosin. These were examined under microscope for histopathological changes such as congestion, haemorrhage, necrosis, inflammation, infiltration, erosion and ulcer and photographs were taken.

RESULTS

Preliminary phytochemical analysis: Preliminary phytochemical analysis showed the presence of phytoconstituents such as flavonoids, tannins, saponins and alkaloids.

Acute toxicity study: During the 14-day study period up to the dose of 5000 mg/kg body weight p.o. for the methanolic extract of A. americana leaves, there were no reported side effects or animal deaths. Therefore, two doses of the extract were chosen for testing the hepatoprotective action against paracetamol-induced toxicity: 100 mg/kg and 200 mg/kg, body weight P.O.

Clinical signs and mortality: The administration of paracetamol and various extracts of Clerodendrum Serratum did not result in any death or illness in the animals over the course of the investigation. The observations made on the cage side did not reveal any clinical symptoms that may be attributed to the toxicity of the substance examined. The rats that were treated with extract did not exhibit any abnormal behaviors, including tremors, convulsions, salivation, diarrhea, lethargy, or any other abnormalities, during the duration of the trial.

Effect of Clerodendrum Serratumextracts on body weight: The weight of the body was measured on day 0 (before to the administration of the dose) and day 8 of the study. On day 8, the percentage change in body weight was determined by using the prior body weight as the basis. A comparison of the body weights of the experimental animals from day 0 to day 8. The administration of paracetamol, which is known to produce hepatotoxicity, resulted in a noteworthy decrease ($P<0.01$) in the total body weight.

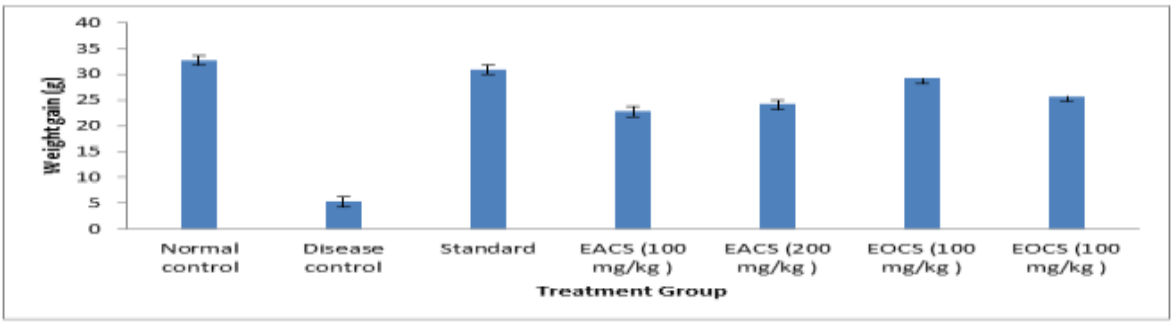

Figure 1: Effect of Clerodendrum Serratumextracts on body weight

Effect of Clerodendrum Serratumextracts on AST, ALT, ALP, and total bilirubin: Wistar rats treated with paracetamol (2 mg/kg p.o.) alone developed significant hepatocellular damage, as evidenced by an increase in serum biomarkers AST, ALT, ALP, and total bilirubin when compared to the control group, according to the findings of the hepatoprotective study of the ethanolic extract of Clerodendrum Serratum. Prior to the administration of paracetamol, rats were pretreated with a ethyl acetate and ethanolic extract of Clerodendrum Serratumseeds at doses of 100 mg/kg, and 200 mg/kg. This resulted in a substantial decrease in the levels of AST, ALT, ALP, and total bilirubin that was nearly equivalent to Silymarin. When administered at a dose of 200 mg/kg compared to 100 mg/kg, the ethanolic extract of Clerodendrum Serratumdemonstrated a dose-dependent action, as indicated by the lowered levels of blood enzymes and total bilirubin.

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The impact on the AST levels in the blood plasma: The AST level is controlled in a dose-dependent manner with the administration of extracts from Clerodendrum Serratum. An extract of Clerodendrum Serratummade with ethanol is more efficient than an ethyl acetate extract in controlling the level of AST. When compared to the standard treated group, the treatment with EOCS (200 mg/kg) was able to control the AST level.

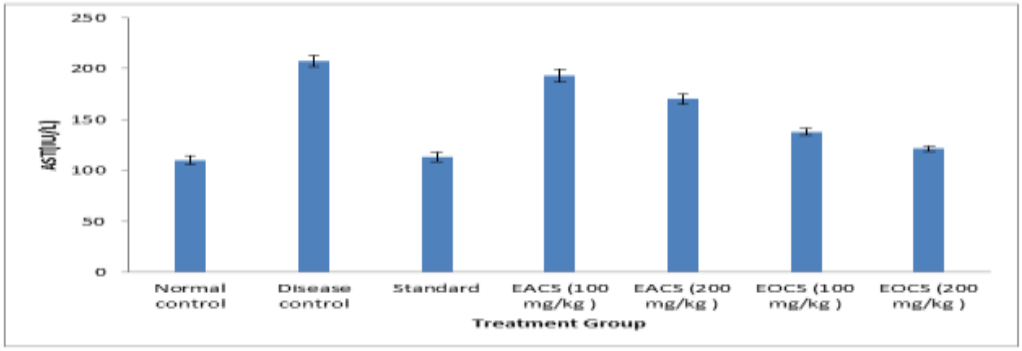

Figure 2: Effect of Clerodendrum Serratumextracts on AST levels in blood plasma ofanimals

Changes in the amount of ALT seen in the blood plasma of animals: A number of different experimental groups were examined to determine the levels of ALT activity that were present in the blood plasma of the animals. The administration of extracts from Clerodendrum Serratum extracts brings about a dose-dependent reduction in the amount of ALT in the body. Ethanol extract of Clerodendrum Serratumwas shown to be more effective in controlling the ALT level as compared to the group that was treated with ethyl acetate extract. The ALT level was controlled by treatment with ethanol extract in a manner that was comparable to that of the standard treated group.

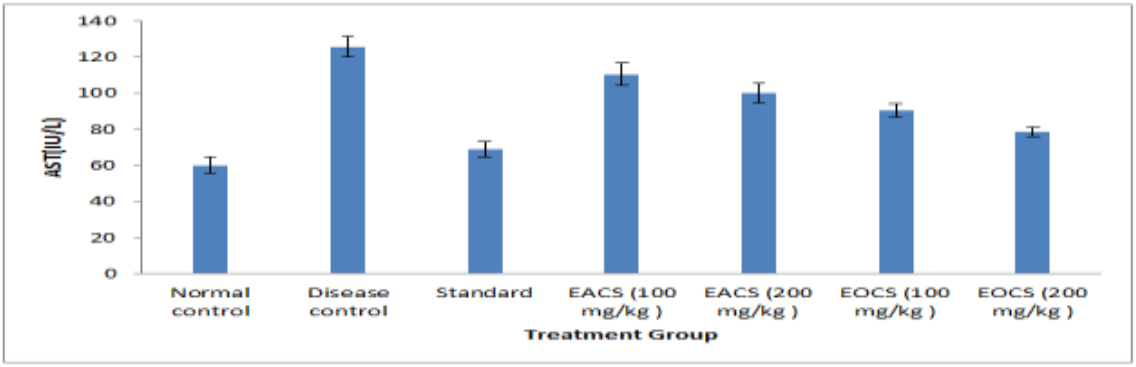

Figure 3: Effect of Clerodendrum Serratum extracts on ALT levels

Influence on the amounts of ALP found in blood plasma: There was a substantial and dose-dependent increase in the ALP level, which was equivalent to the control group. The extracts of Clerodendrum Serratum exhibited lower levels of ALP activity than the control group. In a manner comparable to that of the standard treatment group, the administration of ethanol extracts of Clerodendrum Serratum extracts successfully controls the ALP level. In comparison to the group that was treated with ethyl acetate extract, the ALT level is controlled more effectively by the ethanol extract of Clerodendrum Serratum. This finding suggests that the ethanol extract of Clerodendrum Serratum has the potential to alleviate the blockage of the bile duct that is caused by an excessive amount of paracetamol.

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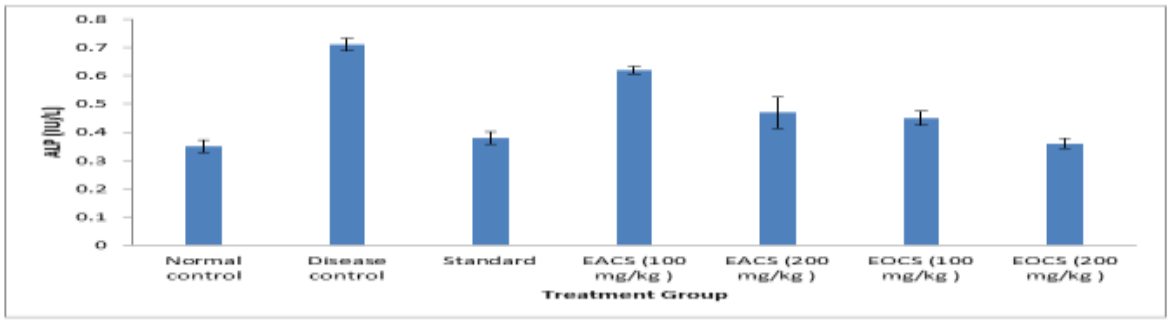

Figure4: Summary of the Clerodendrum Serratumextracts on ALP levels in blood plasma

Influence on the amount of bilirubin: The administration of ethanol extract of Clerodendrum Serratumis more effective in controlling the level of bilirubin. In addition, this indicates that the ethanol extract of Clerodendrum Serratum, when administered at a dosage of 200 mg/kg, are also capable of alleviating the symptoms of hepatotoxicity by providing relief.

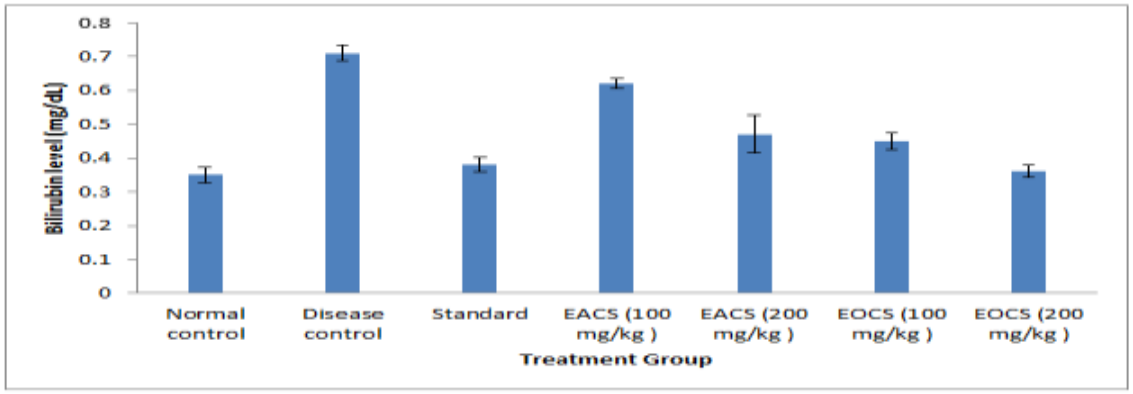

Figure5: Bilirubin level of animals treated with Clerodendrum Serratumextract

Impact on the liver's absolute weight: The absolute weight of the liver in the group that was given ethanol extract of Clerodendrum Serratum at a dose of 200 milligrams per kilogram stayed quite close to the absolute weight of the liver in the control group. There was an increase in liver weight among the disease control group in comparison to the control group. Following treatment with plant extract, the weight of the liver in the group that had been treated with extract was recovered. The liver weight of the treatment group was considerably recovered by the ethanol extract of Clerodendrum Serratumat a dose of 200 mg/kg, which was much higher than the ethyl acetate extract. Furthermore, the administration of ethanol extract resulted in a considerable ($P<0.01$) restoration of the liver weight of the treated group, which was comparable to the weight of the standard treatment group.

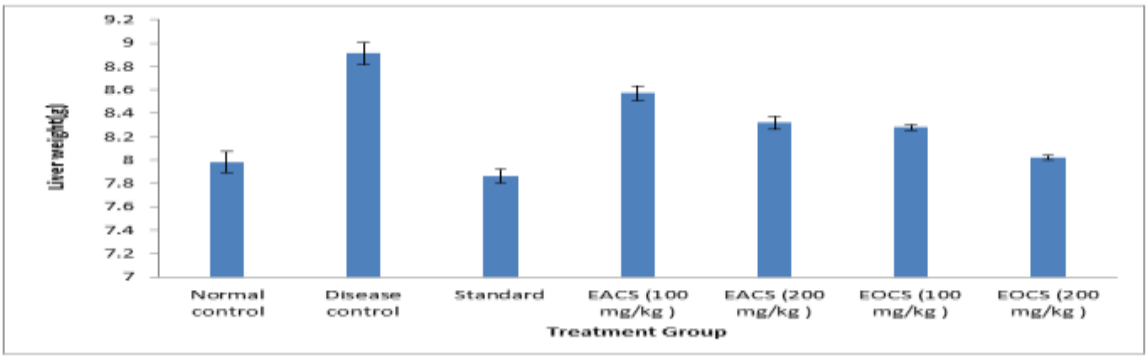

Figure 6: Effect of Clerodendrum Serratumextract on absolute weight of Liver

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Effect on total cholesterol and Triglyceride content levels: When Disease control animals were compared to normal control animals, it was shown that the levels of total cholesterol and plasma triglycerides were much higher in the diseased controlling animals. A comparison was made between the disease control group and the ethanol extract of Clerodendrum Serratum, which was administered at a dose of 200 mg/kg. The results showed that the levels of cholesterol, triglycerides, and LDL reduced dramatically. In light of this, the HDL levels dramatically rose in the groups that were treated with extracts and the standard in contrast to the group that served as the disease control.

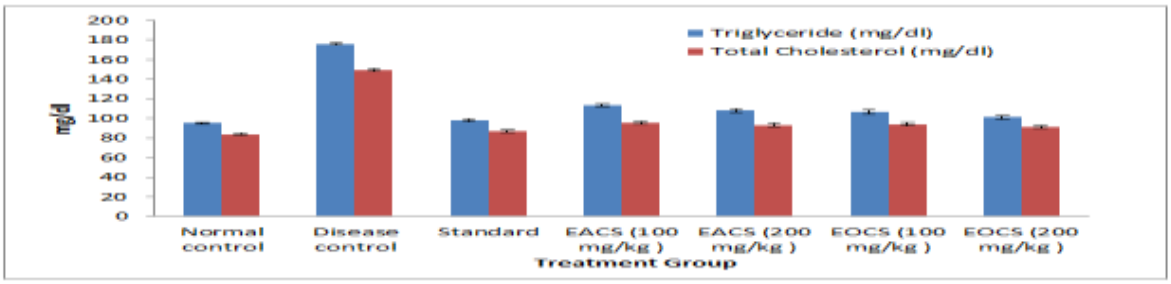

Figure 7: Estimation of the total cholesterol and Triglyceride content

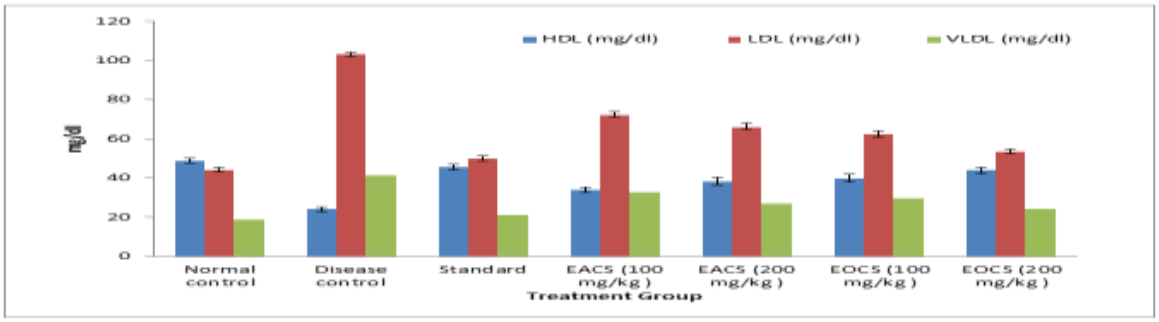

Figure 8: Estimation of the HDL, LDL and VLDL content

Effect on MDA contents in liver: It was found that there was a substantial difference in the levels of malondialdehyde found in normal and disease control samples. The group that was given aethanol extract of Clerodendrum Serratumat a dosage of 200 mg/kg had the highest success rate in terms of recovery. Ethanol extracts have the ability to reduce lipid peroxidation, which is a lipid peroxidation. This finding lends credence to the idea that dietary supplementation with any medicine may lead to an increase in lipid peroxidation. When compared to other extracts, EOCS (200mg/kg) was shown to be the most effective, resulting in a considerable reduction ($P<0.01$) in the MDA content towards normal levels, which was comparable to the standard treated group.

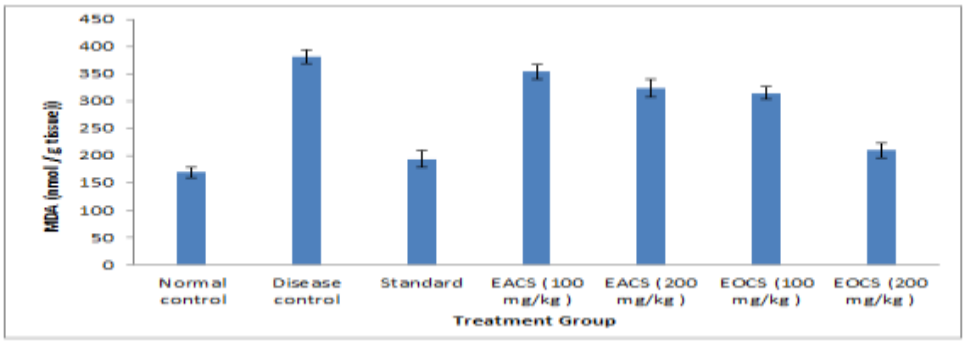

Figure 9: Effect on MDA contents in liver of animals treated with Clerodendrum Serratumextract

Influence on the amount of superoxide dismutase in the liver: A considerable reduction in SOD levels was seen in the animal group that was treated with paracetamol in comparison to the normal control

group. Reversing the condition and greatly increasing the SOD level was accomplished by the standard and EOCS (200 mg/kg) treated group.

The conversion of superoxide radical to hydrogen peroxide is catalyzed by superoxide dismutase (SOD). In spite of the fact that H2O2 is not a radical, it is quickly transformed into a highly reactive hydroxy radical after being subjected to the action of CAT. Therefore, there is a link between two enzymes that is mutually protective of one another. Therefore, the antioxidant defense system is damaged by ROS, which results in a drop in SOD, CAT, and GSH, which ultimately leads to damage to the liver. This is because CAT is responsible for disabling the superoxide radical when it is formed, whereas H2O2 is responsible for inhibiting SOD.

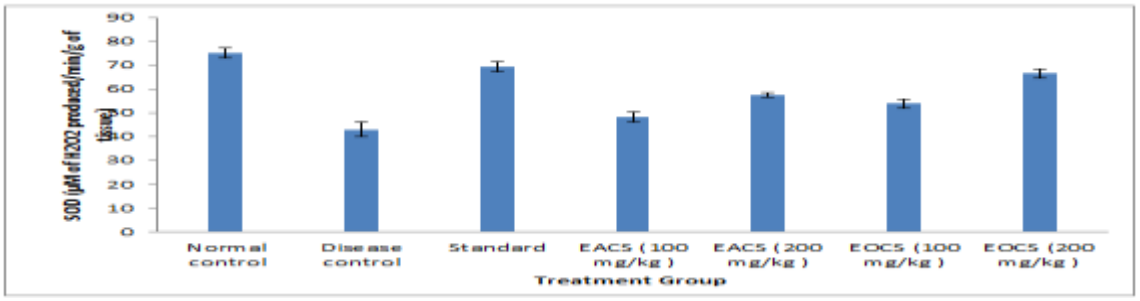

Figure 10: Effect of Clerodendrum Serratumextract on SOD level in liver

With the use of Clerodendrum Serratum extract, the SOD level of the group that had been treated with extract was brought back to normal. In the treatment group, the SOD level was considerably controlled by administering 200 milligrams per kilogram of methanol extract of Clerodendrum Serratum. It was discovered that the ethanol extract of Clerodendrum Serratum(at a dosage of 200 mg/kg) was more efficient than the ethyl acetate extract. It also greatly increased the SOD level and shown a level of SOD that was comparable to that of the standard treatment group.

Effect of Clerodendrum Serratumextract on catalase activity: Catalase activity is reduced by about 53.4% in the animals of disease control group. In a dose-dependent way, different extracts of Clerodendrum Serratumgenerated a rise in catalase activity in contrast to the disease control, which brought the animals closer to return to their normal state. The animals that were given 200 mg/kg of ethanol extracts of Clerodendrum Serratumwere the ones who saw the most successful recovery. The activity level of these animals increased by about 81% compared to the animals that served as controls. Additionally, the catalase activity was discovered to be acquired in animals that were given ethyl acetate extracts; however, the effectiveness of the ethanol extract was shown to be higher.

In comparison to other extracts, the ethanol extract of Clerodendrum Serratum(at a dosage of 200 mg/kg) was shown to be the most effective. It also greatly increased the catalase level and was comparable to the group that was treated with the standard.

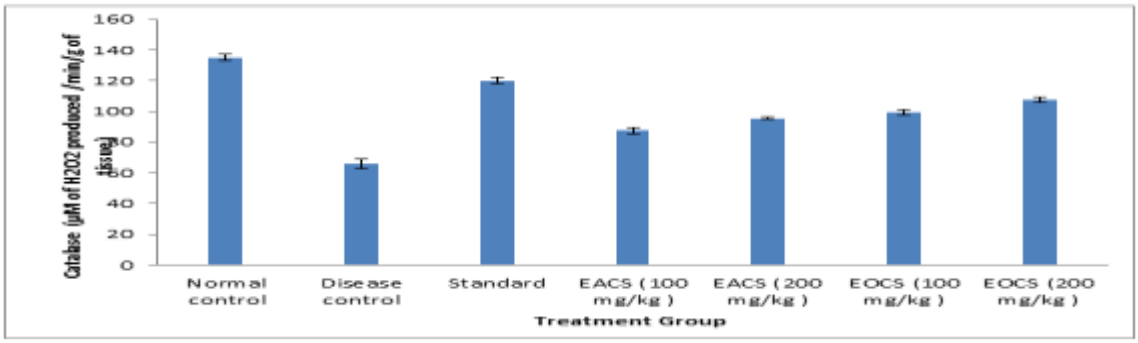

Figure 11: Effect of Clerodendrum Serratumextract on catalase activity

Histopathology Evaluations: At the microscopic level, histopathology was done on the liver tissues of all of the animals in order to assess the modification that occurred as a result of the treatment with paracetamol and the reversal impact of this by different extracts of Clerodendrum Serratumseeds, as well as by silymarin, which is a recognized hepatoprotective chemical.

Clerodendrum Serratumethyl acetateextracts were administered to the group at a dosage of 200 mg/kg, and the results indicated multifocal moderate cytoplasmic vacuolation for the group.Clerodendrum Serratumethanol extracts were administered to the group at a dosage of 200 mg/kg, and the histology of the samples was found to be normal. In the group that was given the hepatoprotective substance silymarin, there was no evidence of any pathologically significant lesions, and the histology was normal. The administration of ethanol extracts of Clerodendrum Serratumto animals provide protection against the hepatotoxicity that is generated by paracetamol.

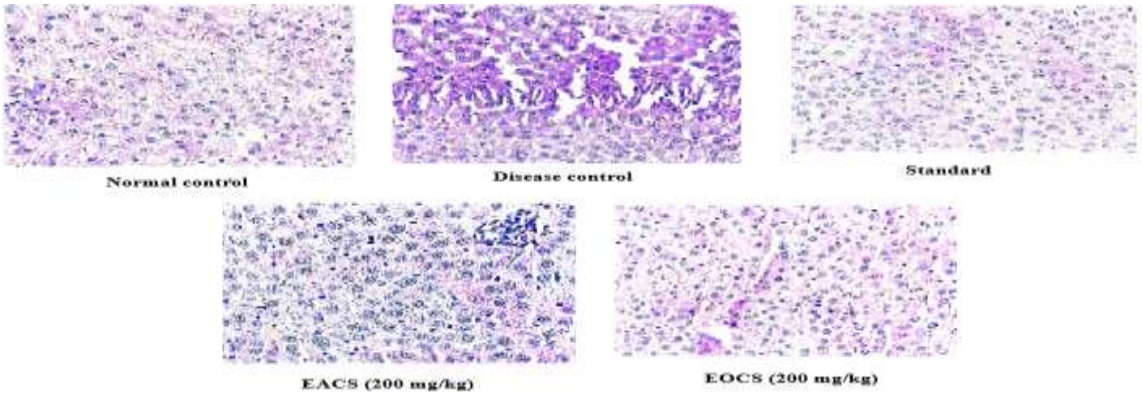

Figure 12: Histopathology Evaluations of liver

DISCUSSION: Prior studies have reported that herbal and medicinal plants substantially contribute to the treatment of cholestatic disorder or hepatocellular insult and liver injury. As significant sources of bioactive compounds with positive effects on health, medicinal plants are receiving a lot of attention. The question of safety and toxicity, however, places significant restrictions on the usage of therapeutic herbs. A common sign of the toxicity of medicinal herbs in vivo is liver injury. The blood biomarkers of liver function include the aminotransferases (ALT, AST), ALP, and bilirubin, with an increase in these markers suggesting hepatic damage. In contrast, total protein and albumin levels are lowered in the presence of hepatic damage.

The little change in blood levels of ALT, AST, and ALP, as well as total bilirubin in liver damage, shows that pretreatment of normal rats with ethyl acetate and ethanolic extract of Clerodendrum Serratumhad no harmful or negative consequences. The current study's findings supported the hypothesis that paracetamol has hepatotoxic effects since it significantly increased the activity of the liver function marker enzymes ALT, AST, ALP, and bilirubin in the serum of rats.

The increased levels of ALT, AST, ALP, and bilirubin in paracetamol-treated rats were dramatically lowered by ethyl acetate and ethanolic extract of Clerodendrum Serratumpretreatment. The stabilizing effect of the Clerodendrum Serratumphytochemical constituent(s) and various active ingredients on the plasma membrane of the hepatocytes, likely caused by the stimulation of hepatocellular protein synthesis and ability to induce microsomal enzymes either by accelerating the excretion of paracetamol or by inhibiting oxidative stress induced by paracetamol, may be responsible for the decreased levels of these serum biomarkers.

The activity of hepatic cells was correlated with blood bilirubin levels. When treated with hepatotoxins (paracetamol), high blood bilirubin content indicates that the liver damage causing a high rate of erythrocyte breakdown. In this investigation, the plant extract resulted in the amount of bilirubin returning to normal levels, suggesting the plant's hepatoprotective activity. The effect was shown to be equivalent to common medications (Silymarin). Results generally imply that the protective action of Clerodendrum Serratum extract normalizes the unbalanced antioxidant system in liver treated with paracetamol.

Histopathological examination of the liver tissues of paracetamol control group represented the presence of marked foci of mononuclear infiltration in the hepatic parenchyma tissue, sinusoid, and around central vein, as well as disorganization of hepatic plates, necrosis, and fatty changes of hepatocytes. Pretreatment with Clerodendrum Serratumethanol extract reversed these alterations.

Clerodendrum Serratumethanol extracts were administered to the group at a dosage of 200 mg/kg, and the histology of the samples was found to be normal. In the group that was given the hepatoprotective substance silymarin, there was no evidence of any pathologically significant lesions, and the histology was normal. The administration of ethanol extracts of Clerodendrum Serratumto animals provide protection against the hepatotoxicity that is generated by paracetamol.

CONCLUSIONS: Clerodendrum Serratumseeds ethanolic extract has hepatoprotective action against paracetamol-induced hepatotoxicity in rats, according to the findings of this study. Regarding the quantitative phytochemical screening and mechanism of action at molecular level, future investigations could be planned. To determine if it is safe for people, studies on long-term toxicity should be employed, and more researches are needed to verify the current findings.In conclusion, the ethanol extract of Clerodendrum Serratumoffered protection from paracetamol-induced liver damage. The protection against liver damage by the ethanol extract of Clerodendrum Serratum was found comparable to silymarin.

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