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Evaluation of Hepatoprotective and Anti-oxidant Activity of *Lemanea fluviatilis* on CCl_4 Induced Hepatotoxicity Rats.

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ABSTRACT

The aim of our present study is to assess the effect of *Lemanea fluviatilis* as a hepatoprotective activity in experimental induced hepatotoxic model in rats. Hepatotoxicity were induced by single dose of carbon tetrachloride which generates free radicals, responsible for the degeneration of liver. Increase concentration of hepatic biochemical markers like Aspartate AminoTransferase (AST), Alanine Amino Transferase (ALT), Alkaline Phosphatase (ALP), Total Bilirubin (TB), Total protein (TP), Triglycerides (TG), Gammaglutamyltransferase (GGT) levels are considered as hepatotoxicities. Administration of Hydroalcoholic extract of *Lemanea fluviatilis* (100, 250, 500 mg/kg) orally for 27 days caused a significant reduction in Lipid peroxidation, Aspartate Aminotransferase, Alanine Aminotransferase, Alkaline Phosphatase, cholesterol and triglyceride levels as compared to animals which received saline water. The hepatoprotective activity was also supported by histopathalogical studies of the liver. These data suggest that *Lemanea fluviatilis* has both hepatoprotective and antioxidants activities.

Keywords: *Lemaneafluviatilis*, Hepatoprotective activities, Carbon tetrachloride, Oxidative stress, Lipid Peroxidaion.

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INTRODUCTION

Liver is one of the largest organ in human body and the chief site for intense metabolism and excretion. It has a surprising role in regulating homeostasis of the body, fight against disease, nutrient supply, energy provision and reproduction, carbohydrate, protein and fat metabolism, detoxification, secretion of bile and storage of vitamin [1]. however, it is continuously exposed to environmental toxins, chemicals like CCl_4, alcohols, auto immune disorder, different antibiotics which leads to various liver diseases such as hepatitis, cirrhosis and liver disease. Oxidative stress plays an important role in chronic complication of liver damage and is postulated to be associated with increased lipid peroxidation [2]. CCl_4 is frequently used to induced liver toxicity in experimental animals. CCl_4 is metabolized by Cytochrome P-450 in endoplasmic reticulum and mitochondria with the formation of CCl_3O^-, a reactive oxidative free radical, which initiates lipid peroxidation [3].

$$CCl_4 \longrightarrow CCl_3O^- + O^-$$

Administration of a single dose of CCl_4 to a rats produces centrilobular necrosis and fatty changes within 24 hrs. The poison reaches its maximum concentration in the liver within 3 hrs of administration. Thus, this free radical (FR) is responsible for the alteration of anti-oxidant defense system such as Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx), Glutathione reductase (GR) and impaired glutathione (GSH) metabolism [4]. Anti-oxidants provide protection to living organism from damage caused by uncontrolled production of ROS concomitant lipid peroxidation, protein damage and DNA strandbreaking [5].

Today, liver diseases are one of the fatal diseases in the world. Modern medicines offer little effects on reduction of hepatic diseases and it is chiefly based on plants preparations which are employed for the treatment of different liver diseases. But there are only few drugs which are available for the treatment of liver disorders.

Herbal drugs provide significant source of hepatoprotective drugs according to an estimate, more than 700 mono and poly-herbal preparations in the form of decoction, tincture, tablets and capsules from more than 100 plants are in clinical use as hepatoprotective [6]. Therefore, many folk remedies from plant origin are tested for its potential antioxidant and hepatoprotective liver damage in experimental animal model.

Lemanea fluviatilis beongs to family lemaneaceae and its locally known as “nungsham”. it has many activities like antidiabetic, hepatoprotective, antioxidant, aphrodisiac, antifertility and antimicrobial activity. Its chemical constituents are amino acid and fatty acid (FA) composition, silver content, total protein, lipids, and carbohydrates of the alga. This alga contains about 24% protein. The most abundant fatty acids were palmitic acid, docosadienoic acid, erucic acid, aspartic acid and oleic acid. The total polyunsaturated fatty acid (PUFA) content obtained was 24.5% with the presence of pharmaceutically important eicosapentaenoic acid (1%)[7-8].

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MATERIALS AND METHODS

Alga collection- The whole alga was collected in the month of December-February from Imphal river. The materials were cleaned thoroughly with fresh water to remove any type of contamination washed alga were air dried in shade.

Preparation of extract- The whole alga were powdered in a grinder and was filled in the Soxhlet apparatus for extraction the whole assembly of the soxhlet apparatus was set up and first the sample powder was defatted by using petroleum ether (60-80˚C) for 72 hrs [9]. after complete defatting, the drug powder were dried at room temperature and extracted with hydroalcohol for 48 hrs. The hydroalcoholic extract of alga were dried at 40˚C in rotary evaporator to produce a semi-solid mass and stored in airtight containers in refrigerator below 10˚C.

Animals- Wistar albino rats of either sex weighing between 160 to 200 gm were selected for the study animal were cages under standard environmental conditions i.e.ambient temperature of 22 ± 2˚C and at 45 to 55% relative humidity for 12 hours each of dark and light cycle [10]. The animals were fed on a standard pellet diet (Lipton rat feed Ltd, Pune) and water ad libitum. All the procedures were performed in accordance with CPCSEA (Committee for the purpose of control and supervision of experimental animals) and approved by the Institutional Animal Ethics Committee (IAEC).

Experimental design for hepatoprotective activity- The rats were divided into five groups contains three animals in each group and received the respective doses as followed (table-1). The suspensions of test samples were administered to rats 1hr, 24 hrs and 48 hrs after single dose of CCl_4 injection. Liver enzymes AST, ALT and ALP levels were measured prior to any drug therapy as well as at the end of the study.

Table-1: Grouping of animals.

Sl no	Animals group	Dose
1	CCl_4 treated	3(ml/kg)
2	CCl_4 plus plant extract	100(mg/kg)
3	CCl_4 plus plant extract	250(mg/kg)
4	CCl_4 plus plant extract	500(mg/kg)
5	Vehicle treated	3ml

Assessment of hepatoprotective activity- In the present study, the hepatoprotective activity was evaluated biochemically and histopathologically. At the end of the study after 48 hrs, all the animals were sacrificed, liver were dissected and preserved in 10% formal saline solution. For Histopathological examination, the sections of hepatic tissues were stain with both hematoxylin-eosin (H-E) stain as well as reticulin stain (Gordon and Sweet's method) and were observed microscopically for Histopathological studies.

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RESULTS

Effect of HAELF on biochemical enzymes levels- In CCl_4 induced hepatotoxicity, the activities of serum hepatic marker enzymes namely aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) and total billirubin level showed a significant increase in CCl_4 treated animals as compared to control group and the total serum protein concentration was significantly lower in CCl_4 treated group (Table II).

Table 2: Effects of HAELF on CCl4 induced hepatotoxicities.

Treatment	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	GGT (IU/L)	T. P (g/dl)	Bilirubin (mg/dl)
Vehicle	69.2 ±3.2	71.21±3.42	191.6±14.3	54.4±0.21	8.24±0.02	1.01±0.04
CCl_4	235.82±3.2	160.79±2.63	320.52±15.6	82.2±0.18	6.1±0.06	4.92±0.13
Extract (100mg/kg)	104.3±5.1	112.24±2.63	217.6±9.2	54.2±0.27	6.9±0.2	1.8±0.21
Extract (250mg/kg)	91.44±3.6	95.72±3.5	206.54±12.4	60.2±0.31	7.8±0.03	2.1±0.25
Extract (500mg/Kg)	72.65±4.1	74.11±3.2	190.6±15.4	64.1±0.29	9.1±0.09	4.1±0.16

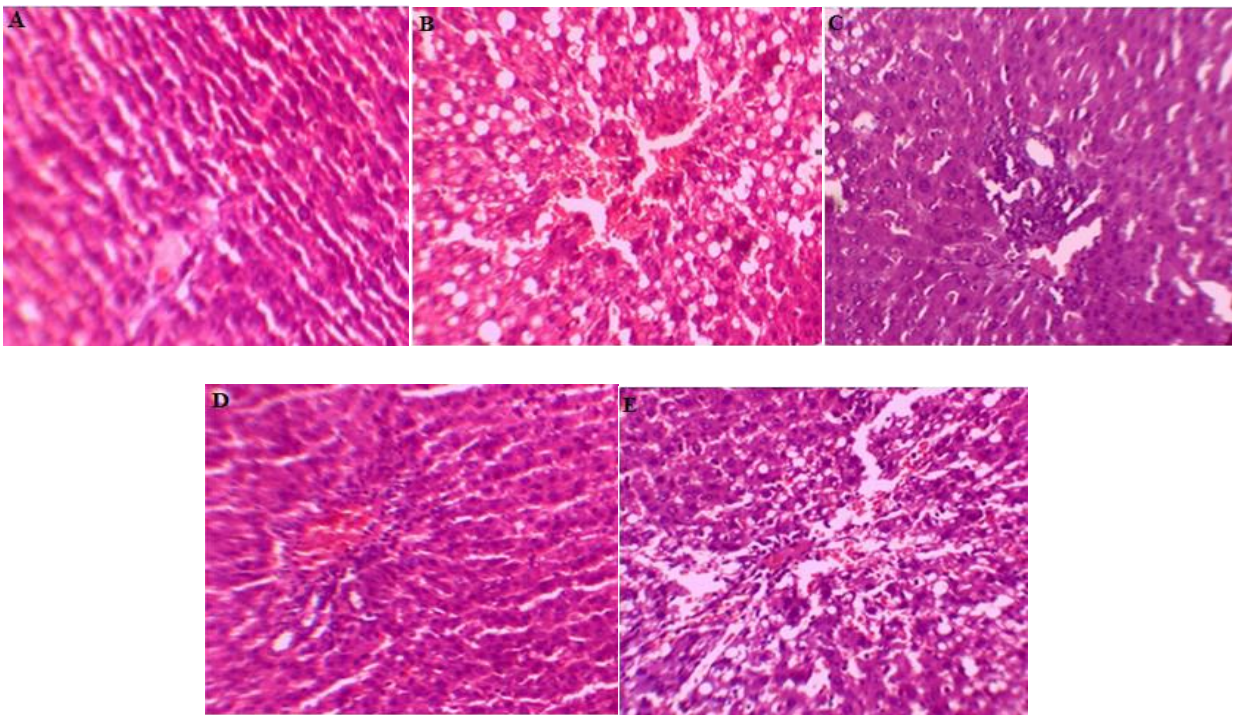

Figure-1; Histopathology of Liver sections of animals (x100). A) Control group, B) CCl_4 treated group C) Treated with HAELF 100 mg/kg, D) Treated with HAELF 250mg/kg, E) Treated with HAELF 500mg/kg.

Administering extract of *lemanea fluviatilis* (500 mg/kg, p.o) significantly reduced the levels of AST, ALT, ALP and total bilirubin level in CCl_4 treated rats and the total serum protein concentration was significantly increased as compared to the group treated with (100 mg/kg) and 250 (mg/kg).those groups which received 100 and 250 (mg/kg,p.o) shows less significance as compared to group which received (500 mg/kg,p.o).

Effect of HAELF on Histopathological studies- The histological examination (Figure-1) shows the effect of *lemanea fluviatilis* extract on CCl_4 induced hepatotoxicity in rats. Control group (A) animals showed a normal hepatic architecture. In CCl_4 treated group (B), severe hepatotoxicity was evidenced by kupffer cell hyperplasia, inflammatory cells, apoptosis, microvascular fatty changes and centrilobular necrosis. Treatment with *lemanea fluviatilis* with different doses (100, 250, 500 mg/kg) reduced abnormalities with less fatty changes.

DISCUSSION

In hepatoprotective experiment, CCl_4 was used to induce hepatotoxicity by metabolic activation. Therefore, it selectively causes toxicity in liver cells maintaining normal metabolic function.

Carbon tetrachloride is accumulated in hepatic parenchyma cells and metabolically activated by the Cytochrome P-450 dependent mixed oxidase in the endoplasmic reticulum to form trichloromethyl free radical ($*CCl_3$) which combined with cellular lipids and proteins in the presence of oxygen to induce lipid peroxidation [11].

These resulted in changes of structures of the endoplasmic reticulum and other membrane, loss of metabolic enzyme activation, elevated level of serum marker enzymes like AST, ALT and ALP reduction of protein synthesis, increased lipid-peroxidation and destruction of Ca^{2+} homeostasis [12].

The impairment in the transport function of the hepatocytes causes the leakage of enzymes from cells due to altered permeability of membrane, which results in decreased levels of AST, ALT and ALP in the hepatic cells and a raised level in serum for such assessment of liver damage by CCl_4 hepatotoxin, the enzyme levels such as AST and ALT is largely considered [13].

Serum ALP and bilirubin levels on the other hand, are related to the function of hepatic cell. Increase in serum level of ALP is due to increased synthesis, in presence of increasing biliary pressure. Bilirubin is one of the most useful clinical clues for the severity of necrosis and its accumulation is a measure of binding, conjugation and excretory capacity of hepatocyte [14].

A reduction in total serum protein observed in the CCl_4 treated group may be associated with the decrease in the number of hepatocytes which in turn may result into decreased hepatic capacity to synthesize protein [15].

In CCl_4-induced hepatotoxicity in rats, our results suggest that the treatment with *lemanea fluviatilis* extract and its different fractions significantly reduced the enhanced level of serum ALT, AST which seem to offer the protection and maintain the functional integrity of hepatic cells. Effective control of bilirubin level and alkaline phosphatase activity by different doses of the extract and its fractions points towards an early improvement in the secretory mechanism of the hepatic cell. The significant raise in protein levels suggests the stabilization of endoplasmic reticulum leading to protein synthesis [16].

These results indicate that the *lemanea fluviatilis* hydroalcoholic extract preserved structural integrity of the hepatocellular membrane and showed dose dependant protective effect.

CONCLUSION

The present study reveals that hepatoprotective activities of HAELF in experimental animal model against CCl_4 induced hepatotoxicity which was proved by the serum marker enzymes and the histopathological changes. Further works are being carried out to isolate and identify the active principle involved in the hepatoprotective activities of this plant extract.

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