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RESEARCH ARTICLE

HEPATOPROTECTIVE ACTIVITY OF HOMALOMENA AROMATICA AGAINST CCL4 INDUCED LIVER INJURY IN SWISS ALBINO MICE

Madhubanti Das[1], Juri Konwar[1] and Jogen Chandra Kalita[1]
Department of Zoology, Gauhati University, Guwahati- 781014, Assam, India.

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Abstract

The *in vivo* hepatoprotective activity of *Homalomena aromatica* was evaluated in Carbon tetrachloride-induced hepatic injury model in Swiss albino mice. Effects of the methanolic rhizome extracts of *Homalomena aromatica* (HAME) at a dose of 200 mg/kg body weight and 500 mg/kg body weight was tested for its efficacy on CCl4 (1ml/kg body weight) induced liver injury. Hepatic toxicity enzyme markers and histological parameters were studied. Daily oral administration of HAME (500 mg/kg) reduced liver toxicity marker enzyme activity significantly as compared to the control. The results were also supported by the histopathological studies. Recovery of the liver tissue was observed in the highest dose (500 mg/kg) of HAME. The progression of liver damage could be inhibited by the protective effect of HAME and the normal architecture of the liver could be preserved. The plant can therefore be a potential therapeutic candidate to treat liver injury.

Introduction:-

The liver is the key organ controlling homeostasis within the body and it is involved in various imperative capacities such as metabolism, secretion and storage. The liver is involved in almost all the biochemical pathways related to development, supply of nutrients, fight against infection and vitality provision. It has incredible capacity to detoxify harmful chemicals and substances and synthesize significant materials. It's typical position and capacities make it the foremost vital organ which in turn makes it susceptible to a number of infections.

Liver diseases have been increasing at an alarming rate over the last few decades and it has become one of the leading causes of illness and mortality globally. Liver disease is affecting millions of people worldwide (Xiao *et al*., 2019). As per the Global Burden of Disease report (2010), more than 2 million deaths were due to significant liver diseases including acute and chronic hepatitis, liver cirrhosis and hepatocellular carcinoma that constituted approximately 4% of all deaths globally (Byass, 2014).

Various toxicants such as Carbon tetrachloride can easily trigger acute liver injury. Carbon tetrachloride (CCl4) is a recognized biohazard. It accumulates in hepatic parenchymal cells and is catalyzed by the phase metabolic enzyme cytochrome P450 2E1 (CYP2E1) to produce unstable free trichloromethyl radicals ($CCl3^-$). CCl4 has been extensively utilized for the experimental induction of acute liver injury resulting from free radical formation that bind to DNA, lipids and proteins (Li *et al*., 2017).

Corresponding Author:- Madhubanti Das
Address:- Department of Zoology, Gauhati University, Guwahati- 781014, Assam, India.

Hepatocytes consists of an antioxidant system comprising of catalase, glutathione, superoxide dismutase, ascorbic acid and tocopherol that provide protection against free radical mediated damage. Disparity between antioxidant defense system and ROS production cause oxidative stress that is responsible for pathophysiological variations allied with many liver ailments such as hepatitis, hepatocellular carcinoma and liver cirrhosis (Abdullah *et al.*, 2017).

Since prehistoric times, medicinal herbs and plants have been utilized worldwide in ethnomedicine and traditional medicine for holistic healing. Phytomedicines have been the conventional genesis of medicines for numerous ailments and various infections for thousands of years. They are re-emerging as an alternative health aid. The North-Eastern region of India being a repository of medicinal herbs and plants; the search for the sources of bioactive compounds in such plants could shed new light on better understanding of the fight against several diseases including many liver ailments. In some indigenous tribal communities of North-East India, wide number of plants and polyherbal formulations in crude forms are utilized for the treatment of various liver ailments and diseases (Sharma and Das, 2018). Alternative herbal medicine can boost the implications of conventional drugs when used appropriately. It is a lot better than the conventional allopathic medications. If used in a specific dose, the plant-derived natural products may not have any side effects. According to the tribal people of this region, medicinal plants work miraculously in certain disease conditions (Asrani *et al*., 2019).

Plants are important sources of medicines. In all countries, plant-based traditional medicines are used for healthcare. According to the World Health Organization (WHO), around 80% of the world's population depends on medicinal plants as their primary health care source. The WHO has reported around 21,000 plants utilized for medicinal purpose. Of these, 2500 species are found in India; 150 species of which are used commercially on a fairly large scale. India is the largest producer of medicinal herbs and therefore, it is called as botanical garden of the world (Seth *et a*l., 2004).

Keeping in view all these aspects, the present study has been undertaken to evaluate and highlight the possible hepatoprotective activity of *Homalomena aromatica* based on traditional ethnomedicinal knowledge-related reports and literature.

Materials And Methods:-

Chemicals

All the chemicals, solvents and drugs were procured from Sigma, Loba Chemie Pvt. Ltd., Merck, Qualigens Fine Chemicals, HiMedia Laboratories Pvt. Ltd., Mumbai, India and other reputed local firms and were of the highest purity and analytical grade. CCl_4 and Silymarin were obtained from Northeast Chemicals. For biochemical estimations, assay kits procured from Beekay Scientific, Panbazar, Assam, India have been used. All chemicals were of analytical grade.

Collection and preparation of plant material

The fresh rhizome of *Homalomena aromatica* were collected from Uttar Pakor Kona, Kamrup district (26.260051º N, 91.605008º E) of Assam, India. The plant was identified in the Department of Botany, Gauhati University, Guwahati, Assam, India. The freshly collected rhizome were thoroughly cleaned, shade dried, segregated and pulverized by mechanical grinder to form a coarse powder. The methanolic extraction of the powdered material was carried out using cold maceration method (Ashraf et al., 2020). 50 g of powdered rhizome were soaked in conical flask containing 500 ml of methanol (1:10 ratio) for 48 hours at room temperature with intermittent stirring. The preparation was then filtered through Whatman No: 1 filter paper. The filtrate was then air dried and stored at 4ºC in the refrigerator for further use.

Animals

Healthy adult Swiss Albino mice of approximately 3 months of age and about 24±5 g body weight was used for the experiment. Animals were obtained from the animal house of the Department of Zoology, Gauhati University. All the animals were maintained under standard laboratory conditions, given normal diet with water *ad libitum* and maintained at standardenvironmental conditions (temperature 25±2 ºC, relative humidity 75±5% and 12 hours light and 12 hours dark cycle). Body weight was recorded throughout the period of experiment.

Toxicity Studies (OECD, Guidance Document on Acute Oral Toxicity Testing, OECD Environment, Health and Safety Publications, 2001)

Acute oral toxicity studies:

In order to evaluate if HAME has any adverse lethal effect on normal mice, acute oral toxicity studies were performed as per OECD 423 guidelines following the method of Lorke et al., 1983. Healthy adult male and female Swiss albino mice

(approximately 03-04 months of age with body weight 24±5 g) were used for the study. All the animals were acclimatized to laboratory conditions for a period of 15 days before commencement of the experiments.

All the animals were randomly divided into six groups having one control group and five treated groups with six animals in each group. Before administration of the test doses, all the animals were fasted overnight with free access to water. Animals were fed orally using gavage. Group I animals received distilled water only and served as control, while groups II, III, IV, V, VI were administered with GPME single doses of 300, 1000, 2000, 3000, 4000 mg/kg body weight respectively. After administration of the plant extract, the animals were maintained on standard animal diet and water. The animals were observed after a 30 minutes interval initially up to 4 h and then over a period of 24 h and then subsequently for the next 14 days for any toxicity and mortality. The animals were observed for any changes in body weight, food intake, water intake, urination, diarrhea, and behavioral profile like tremor, drowsiness, alertness, restlessness, irritability and fearfulness was monitored for the whole duration of the experiment.

Carbon tetrachloride- induced liver injury

For this study the animals were initially divided into five different groups having 6 animals in each group (n=6). Group I was assigned as a Control group and was administered distilled water. Group II was designated as the induction group and was administered with CCl_4 intraperitoneally at a dose of 1 ml/kg 50% CCl_4 suspended in olive oil at 1:1 combination (v/v) biweekly for 3 weeks. Group III was designated as the Reference group and were given Silymarin suspension of 50 mg/kg body weight in distilled water. Group IV and Group V were administered with plant extract suspension orally at a dose of 200 mg/kg body weight and 500 mg/kg body weight for a period of 14 days. All the plant treatment were continued for a period of 14 days.

Group I (Control): Distilled water

Group II (Toxic control): 1 ml/kg 50% CCl_4 suspended in olive oil at 1:1 combination, i.p.

Group III (HAME200): Extract suspension (200 mg/kg) + 1 ml/kg 50% CCl_4

Group IV (HAME500): Extract suspension (500 mg/kg) + 1 ml/kg 50% CCl_4

Group V (Reference group): Silymarin (50 mg/kg) + + 1 ml/kg 50% CCl_4

Collection of blood samples for estimation of biochemical parameters

Animals of all the groups were sacrificed by cardiac puncture after mild diethyl ether anesthesia on the 15th day for estimation of hepatic enzyme levels and hepatic oxidative stress parameters. Blood samples were kept for 30 min, and then centrifuged at 3000 rpm for 15 min. The serum was separated out for estimation of biochemical parameters namely AST, ALT, ALP, total bilirubin, total protein and albumin using the standard kits. All the estimations were carried out according to the manufacturer's instructions of the assay kits. All the biochemical estimations were carried out in triplicate.

Histopathological studies

Livers of sacrificed animals were collected and thoroughly perfused in ice-cold saline. The livers were fixed in 10% Neutral buffered formalin for 48 hrs. The livers were embedded in liquid paraffin following the standard microtomy protocol. 5µ thick sections of paraffin embedded liver were used for staining (Delafield's hematoxylin and eosin stain) following routine histological procedure.

Results and Discussion:-

1. Toxicity studies

1.1. Acute oral toxicity studies

The results of acute oral toxicity test revealed that the oral administration of HAME up to 4000 mg/kg b.wt did not show any lethality or toxic reactions in any of the doses selected until the end of the study. There were no significant differences in the body weight of the treated animals than control till the entire study period (Table 1.1). Administration of HAME did not show any alterations in behavior, water intake, food intake, body weight, diarrhea, urination, tremor, changes in skin hair, general physique, comma and mortality. No mortality was recorded throughout the period of observation of acute toxicity.

Table 1.1:- Body weight (g) of mice during acute toxicity study treated with different doses of methanolic extract of rhizome of *Homalomena aromatica*. Values are expressed as mean ± SEM, n=6 animals/group. The values are statistically significant at $p< 0.05$ compared with control group and analyzed by one way ANOVA.

Treatment groups	Initial body weight (g)	Final body weight (g)
Control	26.7±1.85	28.1±1.35

300 mg/kg b.w treated group	24.3±1.11	25.2±1.76
1000 mg/kg b.w treated group	27.8 ± 1.63	29.2 ± 1.19

2000 mg/kg b.w treated group	25.1 ± 1.89	26.3 ± 1.72
3000 mg/kg b.w treated group	26.5±1.44	27.9±1.64
4000 mg/kg b.w treated group	27.6±1.34	29.1±1.28

2. *In-vivo* hepatic oxidative stress parameters on CCl4 induced liver injury

2.1. Changes in level of serum ALT

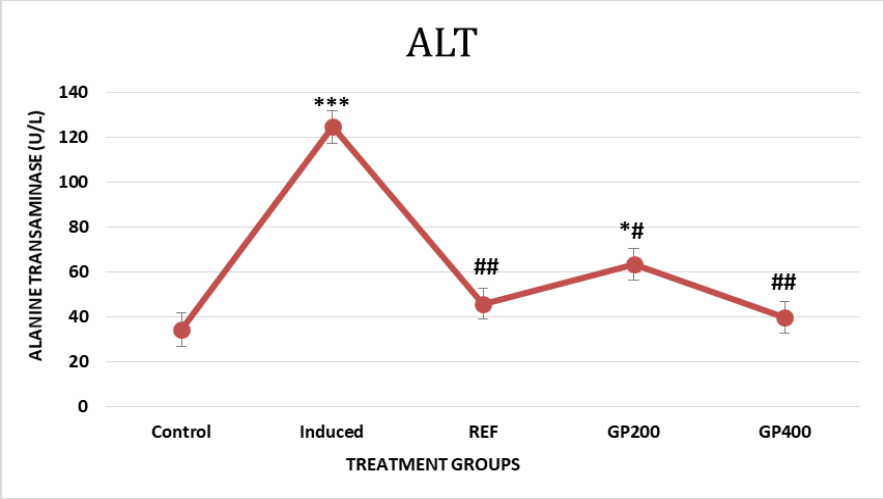

Fig 2.1:- Changes in serum level of ALT (U/L) in Swiss albino mice after CCl4 treatment. Values are expressed as mean ± SEM, (n=6). The values are statistically significant at*p<0.05; **p<0.01; ***p<0.001 as compared to the control group and #p<0.05; ##p<0.01; ###p<0.001 as compared to the induced group (One way ANOVA).

2.2. Changes in level of serum AST

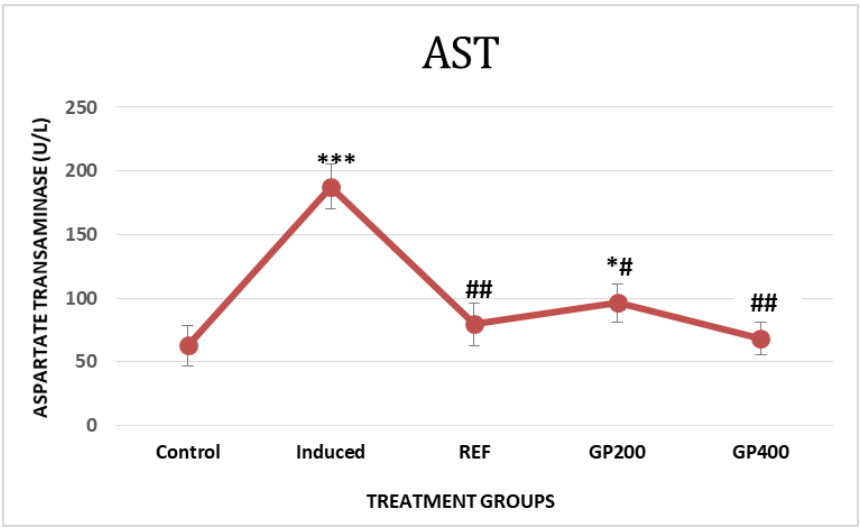

Fig 2.2:- Changes in serum level of AST (U/L) in Swiss albino mice after CCl4 treatment. Values are expressed as mean ± SEM, (n=6). The values are statistically significant at *p<0.05; **p<0.01; ***p<0.001 as compared to the control group and #p<0.05; ##p<0.01; ###p<0.001 as compared to the induced group (One way ANOVA).

2.3. Changes in level of serum ALP

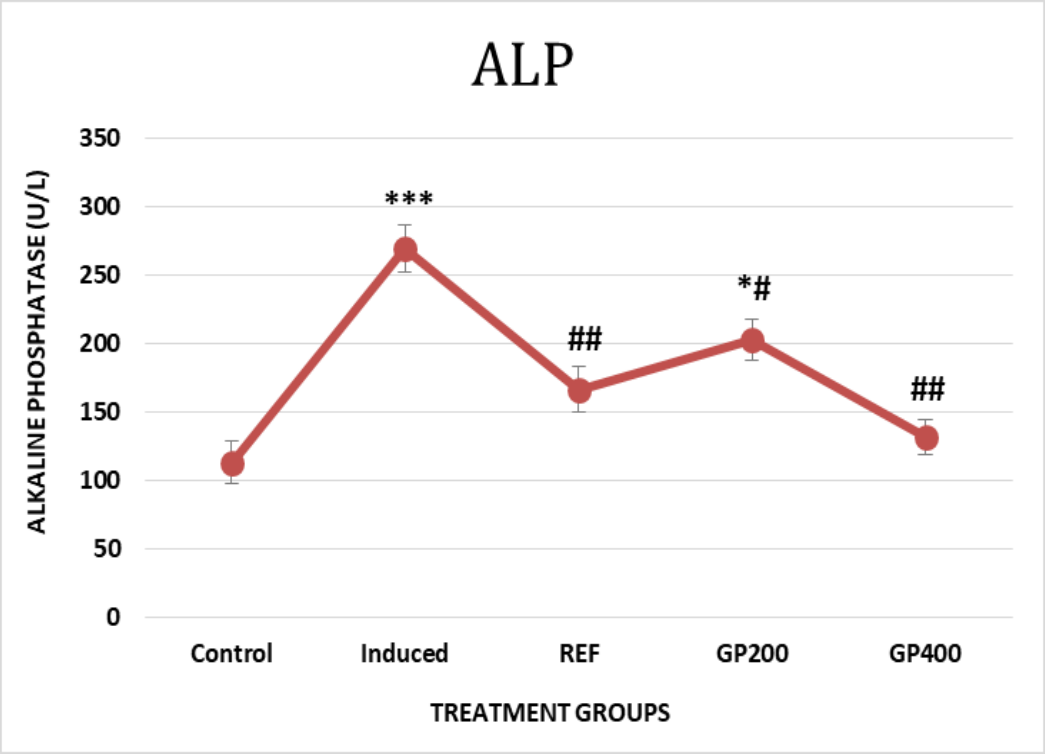

Fig 2.3:- Changes in serum level of ALP (U/L) in Swiss albino mice after CCl4 treatment. Values are expressed as mean ± SEM, (n=6). The values are statistically significant at *p<0.05; **p<0.01; ***p<0.001 as compared to the control group and #p<0.05; ##p<0.01; ###p<0.001 as compared to the induced group (One way ANOVA).

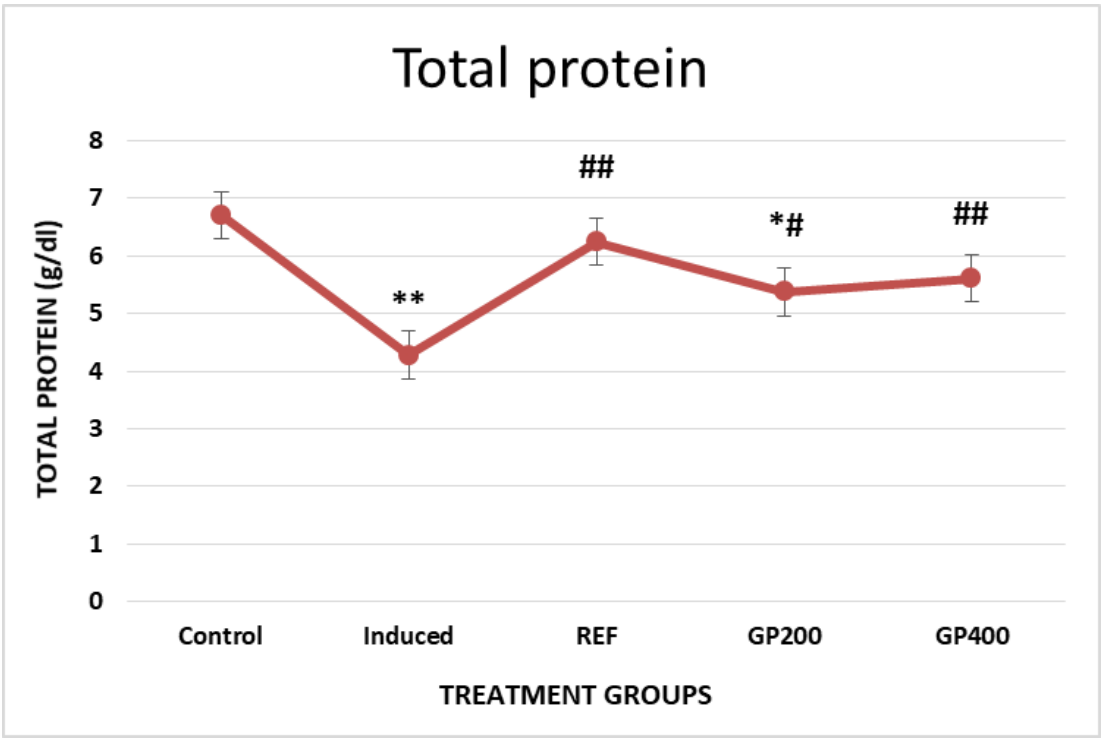

Fig 2.4:- Changes in serum level of total protein (g/dl) in Swiss albino mice after CCl4 treatment. Values are expressed as mean ± SEM, (n=6). The values are statistically significant at *p<0.05; **p<0.01; ***p<0.001 as compared to the control group and #p<0.05; ##p<0.01; ###p<0.001 as compared to the induced group (One way ANOVA).

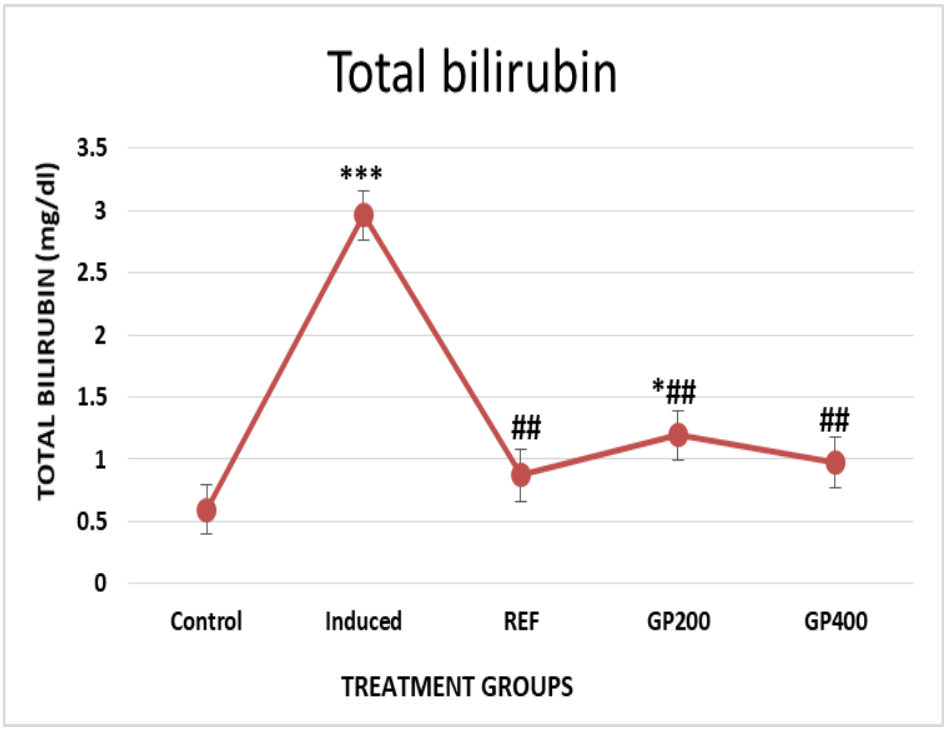

Fig 2.5:- Changes in serum level of total bilirubin (mg/dl) in Swiss albino mice after CCl4 treatment. Values are expressed as mean ± SEM, (n=6). The values are statistically significant at *p<0.05; **p<0.01; ***p<0.001 as compared to the control group and #p<0.05; ##p<0.01; ###p<0.001 as compared to the induced group (One way ANOVA).

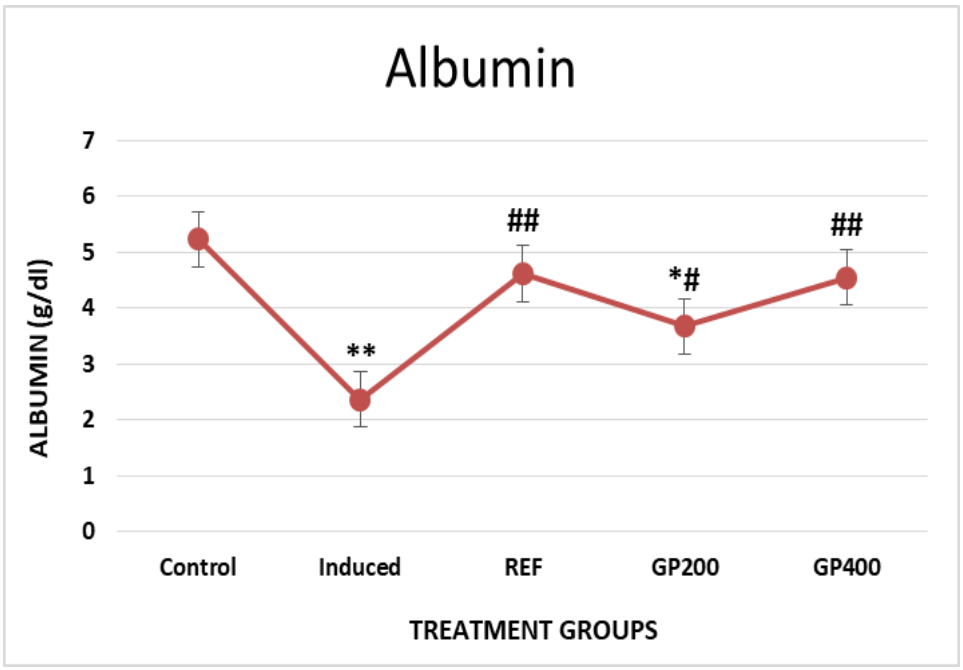

Fig 2.6:- Changes in serum level of albumin (g/dl) in Swiss albino mice after CCl4 treatment. Values are expressed as mean ± SEM, (n=6). The values are statistically significant at *p<0.05; **p<0.01; ***p<0.001 as compared to the control group and #p<0.05; ##p<0.01; ###p<0.001 as compared to the induced group (One way ANOVA).

The biochemical results showed a marked increase of all biochemical parameters i.e. ALT, AST, ALP and total bilirubin after administration of 1 ml/kg 50% CCl4 to induce hepatic damage. As shown in Figure 2.1, Figure 2.2 and, the concurrent treatment of methanolic rhizome extract of HAME at a dose of 500 mg/kg significantly (p<0.05) decreased the

elevation of AST and ALT by the CCl4 intoxication and thus provides satisfactory hepatoprotection in a dose dependent manner which was comparable to the effect of the reference drug Silymarin. Notably, HAME administration at a dose of 500 mg/kg significantly ($p<0.05$) reduced the levels of ALP (Figure 2.3) and total bilirubin (Figure 2.5), which was non-significant to those in the normal and silymarin-treated groups. In the present study, administration of CCl4 to the animals before treatment by HAME significantly decreased the serum total protein levels and albumin levels. The administration of methanolic rhizome extract of HAME at a dose of 500 mg/kg significantly ($p<0.05$) increased the level of total protein levels (Figure 2.4) and albumin levels (Figure 2.6). These findings suggested that HAME significantly reduced the hepatic intoxication induced by CCl4.

3. Histopathology studies of liver tissue

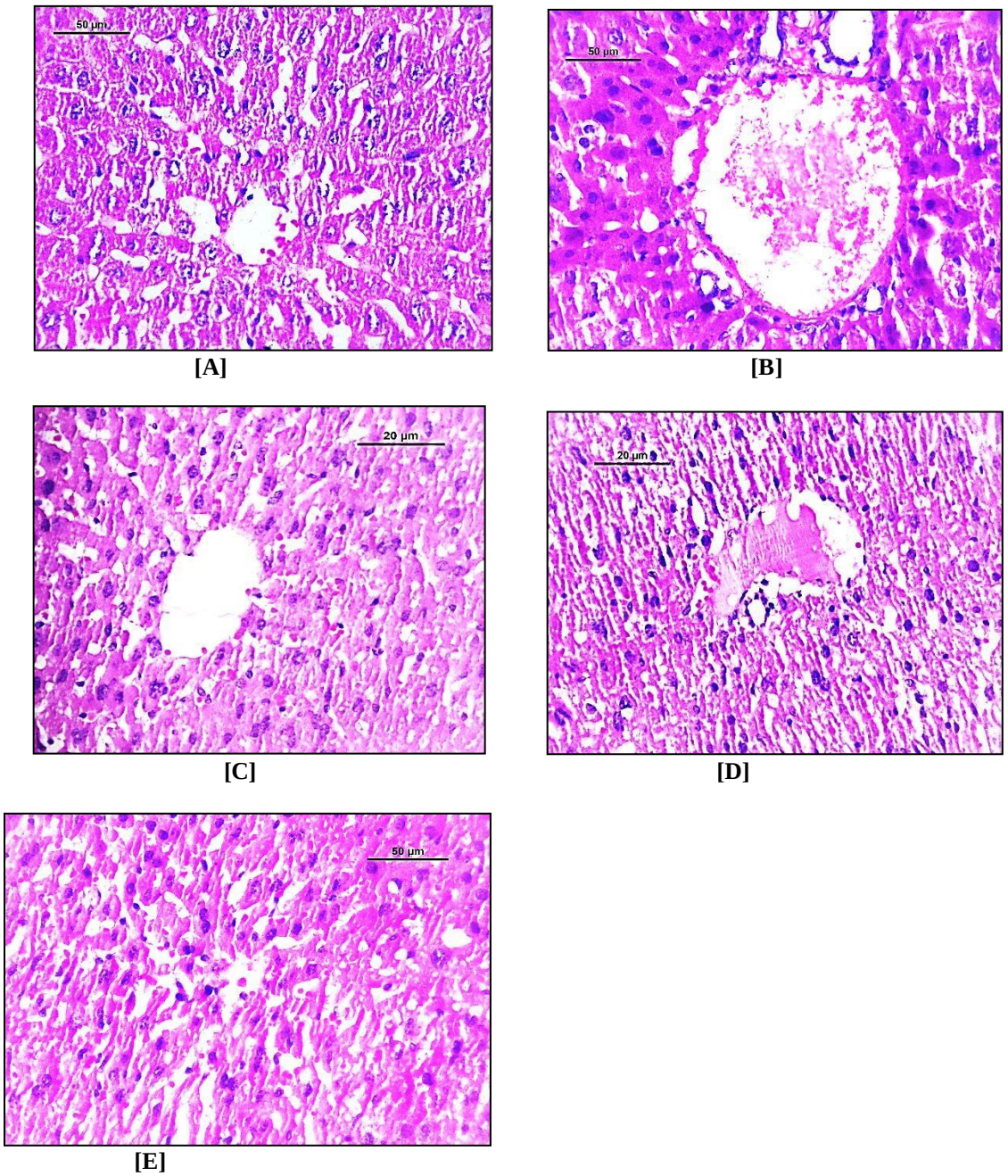

Fig 3.1:- Photographs of histological alterations of liver tissue after CCl4 intoxication. [A] is the normal control group, [B] is the CCl4 treated group, [C] is the Silymarin (50 mg/kg body weight) treated group, [D] is the low dose HAME treated group (200 mg/kg body weight), [E] is the high dose HAME treated group (500 mg/kg body weight).

The histopathological results also established the authenticity of the biochemical findings. The photomicrographs of mice liver give a clear view of the gradual recovery from CC14 induced hepatic damage. In Fig 3.1[A], the normal control group presents normal liver architecture; hepatocytes are very well arranged, central vein without any alterations. The livers of mice of CC14 induced group as seen in Fig 3.1[B], have extensive fatty changes, spotty and hyaline necrosis, extensive accumulation of connective tissue resulting in formation of continuous fibrotic septa, noticeable alteration in the central vein and inflammation in comparison to the normal control group. Livers of animals treated with 200 mg/kg of HAME shows very good protection as seen in Fig 3.1[D] as compared to that of the animals in the induced group. Very satisfactory protection was visible in the liver slides of mice treated with 500 mg/kg of HAME as seen in Fig 3.1[E] because there is no inflammation or necrosis or fatty deposition and the central vein architecture is about normal. The histopathological plates of the reference drug silymarin treated liver (Fig 3.1[C]) attribute near normal state of liver in the 28 days study period as compared to the animals in the control group. It is evident from the results of this study that HAME has a strong *in vivo* hepatoprotective activity.

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