Assessment of Hepatoprotective and Antioxidant Activity of *Achyranthes Aspera* Against Ccl_4 Induced Hepatic Damage in Swiss Albino Mice

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Abstract- The *in vivo* hepatoprotective and antioxidant effect of *Achyranthes aspera* was evaluated in carbon tetrachloride-induced hepatic injury model in Swiss albino mice. Effects of the methanolic leaf extracts of *Achyranthes aspera* (AAME) at a dose of 200 mg/kg body weight and 500 mg/kg body weight was tested for its efficacy on CCl_4 (1ml/kg body weight) induced liver injury. Biochemical, histological and hepatic oxidative stress parameters were studied. Daily oral administration of AAME (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 AAME. The progression of liver damage could be inhibited by the antioxidant activities of AAME and the normal architecture of the liver could be preserved. The plant can therefore be a potential therapeutic candidate to treat liver injury.

Keywords: Hepatoprotective, Antioxidant, *Achyranthes aspera*, Liver injury, Silymarin, Medicinal plant.

1.INTRODUCTION

The liver is the key organ directing homeostasis within the body. It is involved with nearly all the biochemical pathways related to development, fight against infection, supply of nutrients and vitality provision. It is involved in various imperative capacities such as metabolism, secretion and storage. It has incredible capacity to detoxify harmful chemicals and substances and synthesize valuable materials. It's typical position and capacities make it the foremost basic organ but moreover inclined to number of infections. But in the present scenario, liver disease is affecting millions of people worldwide (Xiao *et al.,* 2019).

Over the last few decades, liver diseases have been increasing at an alarming rate and has turned out to be one of the leading causes of mortality and illness globally. As per the Global Burden of Disease report (2010), more than 2 million deaths were resulted from significant liver diseases including acute and chronic hepatitis, liver cirrhosis and hepatocellular carcinoma, which constituted for approximately 4% of all deaths globally (Byass, 2014).

Acute liver injury can be easily triggered by various toxicants such as Carbon tetrachloride (CCl_4), a recognized biohazard that accumulates in hepatic parenchymal cells and is catalyzed by the phase metabolic enzyme cytochrome P450 2E1 (CYP2E1) to produce unstable free trichloromethyl radicals (CCl^{-}_{3}) has been extensively utilized for the experimental induction of acute liver injury resulting in the in formation of free radicals that bind to DNA, lipids and proteins (Li *et al.,* 2017).

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

India is a repository of extraordinary geographical and climatic factors and is gifted with an abundant and diverse flora of medicinal herbs and plants since the Vedic period. India is one of the largest producer of medicinal herbs. Approximately 20,000 medicinal plants have been recorded in India and around 7000 to 7500 plants are used by the traditional practitioners in India for curing different ailments (Pandey *et al.,* 2013).

Medicinal plants and herbs have been utilized since prehistoric times in traditional medicine and

ethnomedicine worldwide for holistic healing. Phytomedicines are re-emerging as an alternative health aid and have been the conventional genesis of medicines for numerous ailments and infections for thousands of years. The Northeastern region of India is a repository of medicinal plants and herbs. The search for the sources of bioactive compounds of such plants could cast new light on the better understanding of the fight for many diseases including various liver ailments. In the Northeastern region of India, especially in some tribal communities, a vast number of plants and polyherbal formulations in crude forms are used for the treatment of various liver diseases (Sharma and Das, 2018).

Alternative medicine can intensify the implications of conventional drugs if used appropriately and is a lot better than the conventional allopathic medications. The plant derived natural products may not have any side effects if used in a specific dose and according to some of 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. World Health Organization (WHO) estimated that 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 are used for medicinal purpose. Of which 2500 species are in India, among these 150 species are used commercially on a fairly large scale. In a world, India is the largest producer of medicinal herbs and is called as a botanical garden of the world (Seth *et al.,* 2004).

Keeping in view, the present study has been undertaken based on ethnomedicinal and traditional knowledge based reports and literature to evaluate the possible hepatoprotective and antioxidant effect of *Achyranthes aspera*.

2.MATERIALS AND METHODS

2.1 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 local medical store (Micro Labs Limited, Bengaluru, India). For biochemical estimation, test kits from Aspen Laboratories and Crest Biosystems, Goa, India have been used. All chemicals were of analytical grade.

2.2 Collection and preparation of plant material

The fresh leaves of *Achyranthes aspera* were collected from Bahana, Kamrup district (26.266332^o N, 91.609765^o E) of Assam, India. The plant was identified in the Department of Botany, Gauhati University, Guwahati, Assam, India. The freshly collected leaves 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 leaves 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 4^{o}C in the refrigerator for further use.

2.3 Experimental Animals

Healthy adult Swiss Albino mice of approximately 3 months of age and about 24±5 g body weight were 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 standard environmental conditions (temperature 25±2 ^{o}C, relative humidity 75±5% and 12 hrs light and 12 hrs dark cycle). Body weight was recorded throughout the period of experiment.

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

2.4.1 Acute oral toxicity studies

In order to evaluate if AAME has any adverse lethal effect on normal mice, acute oral toxicity studies was 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 group 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 minute 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.

2.5 Carbon tetrachloride- induced hepatic damage

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 CCI_4 intraperitoneally at a dose of 1 ml/kg 50% CCI_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% CCI_4 suspended in olive oil at 1:1 combination, i.p.

Group III (Test group): Extract suspension (200 mg/kg) + 1 ml/kg 50% CCI_4

Group IV (Test group): Extract suspension (200 mg/kg) + 1 ml/kg 50% CCI_4

Group V (Reference group): Silymarin (50 mg/kg) + + 1 ml/kg 50% CCI_4

2.6 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, superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GSH) 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.

2.7 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.

3. RESULTS AND DISCUSSION

3.1 Toxicity studies

3.1.1 Acute oral toxicity studies

The results of acute oral toxicity test revealed that the oral administration of AAME 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 3.1). Administration of AAME 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 3.1: Body weight (g) of mice during acute toxicity study treated with different doses of methanolic extract of leaf of *Achyranthes aspera*. 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	23.4±1.27	25.3±1.44
300 mg/kg b.w treated group	25.1±1.78	27.2±1.96
1000 mg/kg b.w treated group	25.1 ± 1.1	26.8 ± 1.34
2000 mg/kg b.w treated group	25.8 ± 1.32	26.7 ± 1.37
3000 mg/kg b.w treated group	26.1±1.12	27.2±1.74
4000 mg/kg b.w treated group	23.6±1.77	25.1±1.2

3.2 *In-vivo* hepatic oxidative stress parameters on CCl_4 induced liver injury

Administration of CCl_4 resulted in a significant reduction ($p<0.05$) in hepatic CAT levels, GSH and SOD levels as compared with the normal control group (Table 3.2). AAME treatment (200 mg/kg and 500 mg/kg) produced a marked and dose dependent elevation in hepatic GSH. Notably, hepatic GSH content in the group treated with 500 mg/kg of AAME showed no significant difference from the normal control group. The results of the present study also indicated that AAME at a dose of 500 mg/kg was the most effective in reviving the alterations caused by CCl_4 intoxication. It is evident from the results of this study that AAME has a strong *in vivo* antioxidant activity.

Table 3.2: Changes in hepatic oxidative stress parameters after CCl_4 induced liver injury. Values are expressed as mean ± SEM, n=6 animals/group. 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).

Treatment groups	SOD (U/mg protein)	Catalase (U/mg protein)	GSH (µmol/mg protein)
Control	73.6±1.53	38.1±1.72	30.3±1.35
Induced	31.5±1.74 **	19.4±1.34 **	17.2±1.8 **
Plant 200 mg/kg	42.9± 1.33 *#	26.5± 1.7 *#	21.1± 1.63 *#
Plant 500 mg/kg	58.7± 1.17 ##	31.4± 1.38 ##	25.8± 1.79 ##
Reference	63.6±1.94 *##	34.7±1.44 ##	27.8±1.26 ##

3.3 *In-vivo* hepatic oxidative stress parameters on CCl_4 induced liver injury

3.3.1 Changes in level of serum ALT

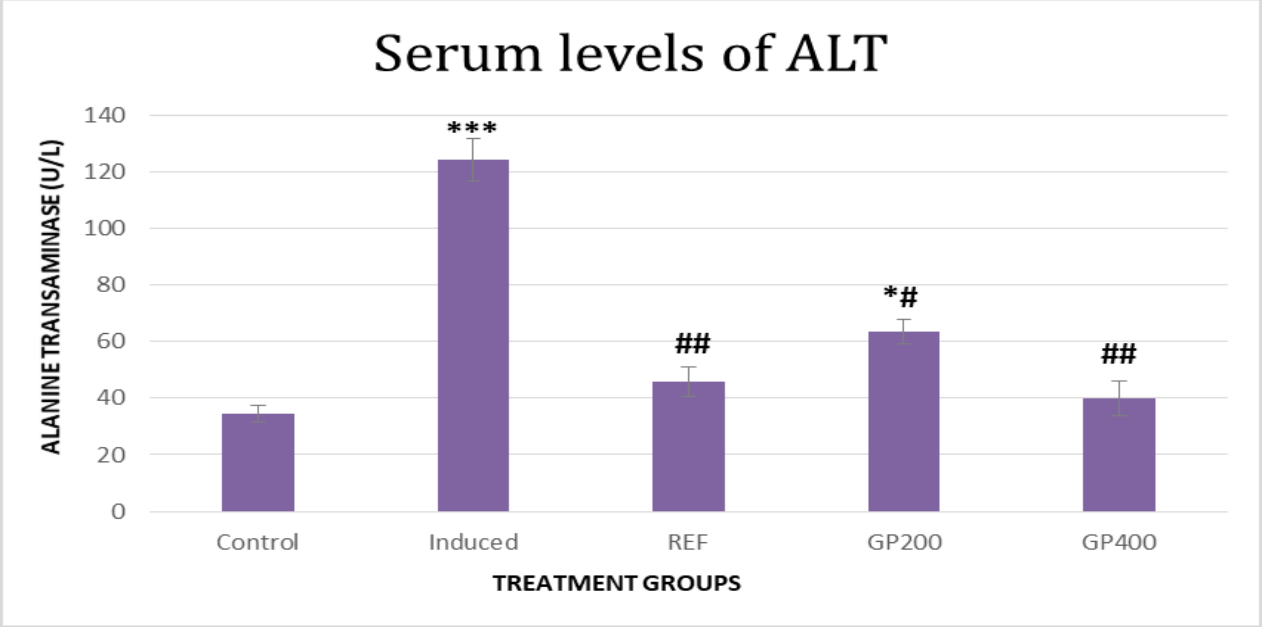

Fig 3.1: Changes in serum level of ALT (U/L) in Swiss albino mice after CCl_4 treatment. Values are expressed as mean ± SEM, (n=6). The values are statistically significant at *p<0.05; **p<0.01; ***p<0.001 as compared to the control group and #p<0.05; ##p<0.01; ###p<0.001 as compared to the induced group (One way ANOVA).

3.3.2 Changes in level of serum AST

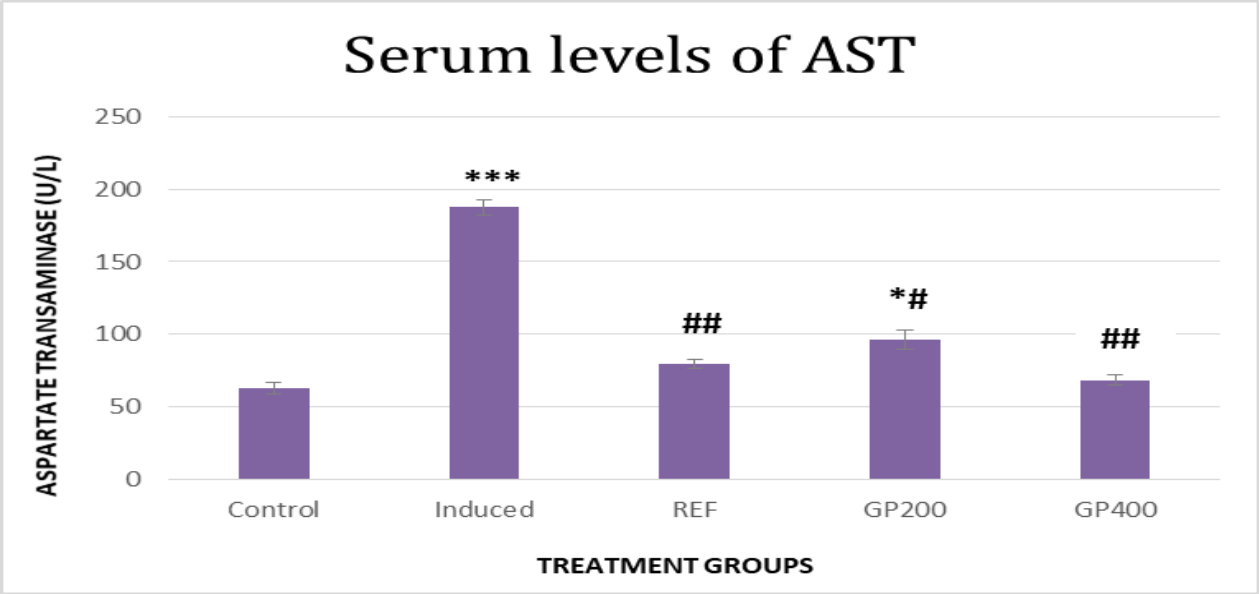

Fig 3.2: Changes in serum level of AST (U/L) in Swiss albino mice after CCl_4 treatment. Values are expressed as mean ± SEM, (n=6). The values are statistically significant at *p<0.05; **p<0.01; ***p<0.001 as compared to the control group and #p<0.05; ##p<0.01; ###p<0.001 as compared to the induced group (One way ANOVA).

3.3.3 Changes in level of serum ALP

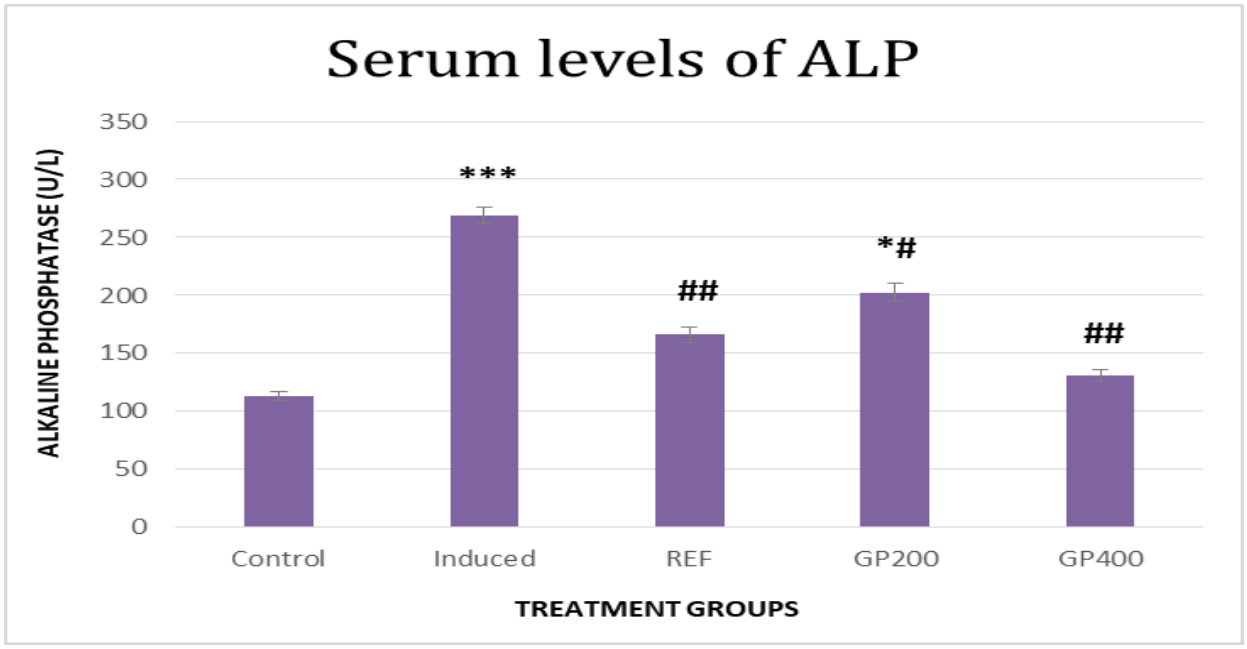

Fig 3.3: Changes in serum level of ALP (U/L) in Swiss albino mice after CCl_4 treatment. Values are expressed as mean ± SEM, (n=6). The values are statistically significant at *p<0.05; **p<0.01; ***p<0.001 as compared to the control group and #p<0.05; ##p<0.01; ###p<0.001 as compared to the induced group (One way ANOVA).

The biochemical results showed a marked increase of all biochemical parameters i.e. ALT, AST and ALP after administration of 1 ml/kg 50% CCI_4 to induce hepatic damage. As shown in Figure 3.1, Figure 3.2 and, the concurrent treatment of methanolic leaf extract of AAME at a dose of 500 mg/kg significantly ($p<0.05$) decreased the elevation of AST and ALT by the CCI_4 intoxication and thus provides satisfactory hepatoprotection in a dose dependent manner which was comparable to the effect of the reference drug Silymarin. Notably, AAME administration at a dose of 500 mg/kg significantly ($p<0.05$) reduced the levels of ALP (Figure 3.3), which was non-significant to those in the normal and silymarin-treated groups. These findings suggested that AAME significantly reduced the hepatic intoxication induced by CCl_4.

3.4 Histopathology studies of liver tissue

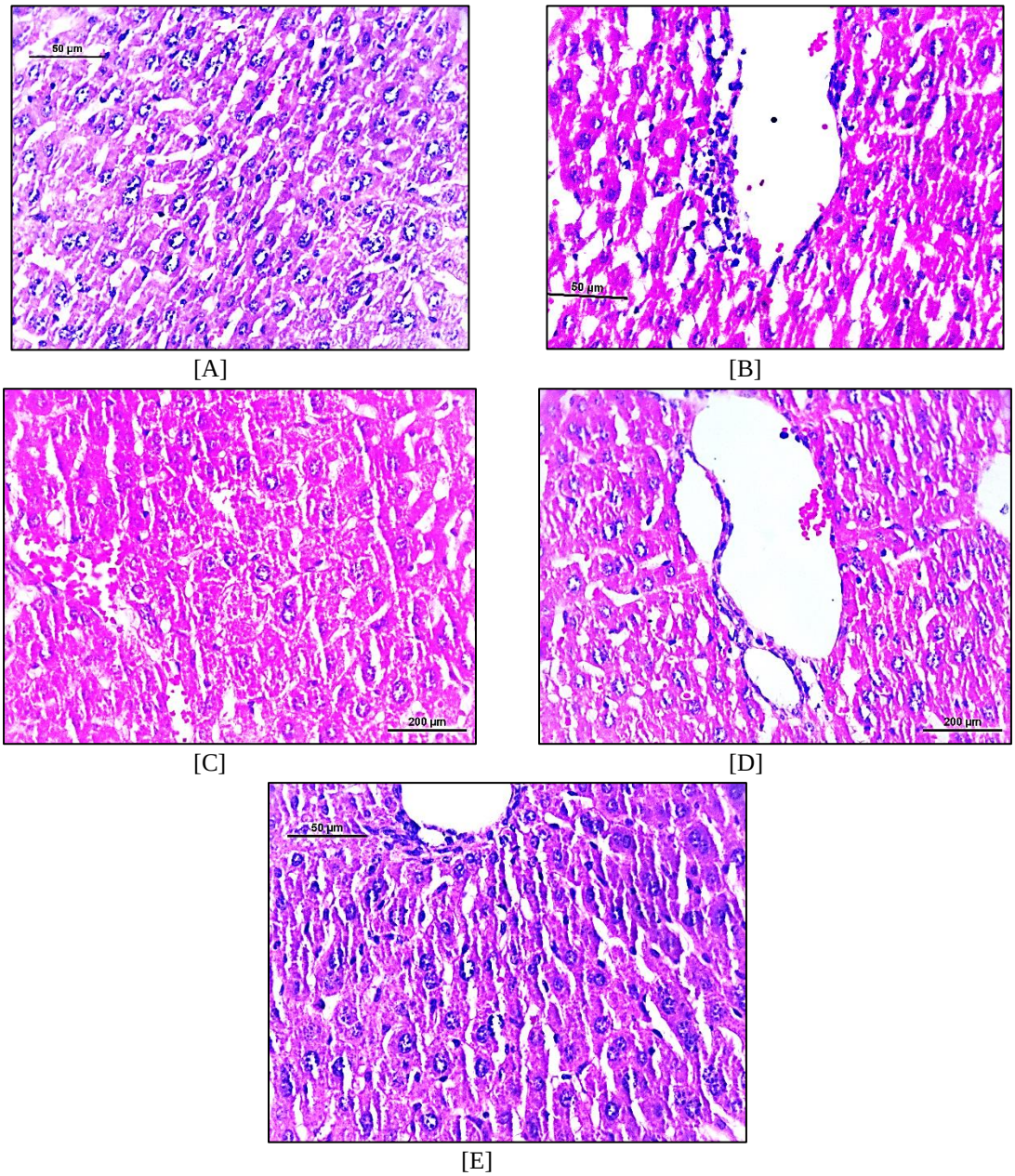

Fig 3.4: Photographs of histological alterations of liver tissue after CCl_4 intoxication. [A] is the normal control group, [B] is the CCl_4 treated group, [C] is the Silymarin (50 mg/kg body weight) treated group, [D] is the low dose AAME treated group (200 mg/kg body weight), [E] is the high dose AAME 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 $CC1_4$ induced hepatic damage. In Fig 3.4[A], the normal control group presents normal liver architecture; hepatocytes are very well arranged, central vein without any alterations. The livers of mice of $CC1_4$ induced group as seen in Fig 3.4[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 AAME shows very good protection as seen in Fig 3.4[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 AAME as seen in Fig 3.4[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 3.4[C]) attribute near normal state of liver in the 28 day study period as compared to the animals in the control group. It is evident from the results of this study that AAME has a strong *in vivo* antioxidant and hepatoprotective activity.

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