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Antioxidant and Anti Lipid Peroxidation Activities of *Annona reticulata* Leaf Extract Against CCl_4-induced Liver Injury in Rats

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Abstract : The present study was aimed to evaluate the effect of ethanol extract of *Annona reticulata* leaves on antioxidant enzymes and lipid profile of CCl_4- induced liver toxicity in rats. The experimental liver injury was induced in animals by CCl_4 (2ml/kg b.w. i.p.) injection and treatment with the dose of (200mg/kg b.w.) was continued for 30 days. At the end of treatment period, oxidative stress parameters like lipid peroxidation by-products; enzymatic antioxidants such as superoxide dismutase (SOD), reduced glutathione (GSH) and catalase (CAT) were calculated in the liver and the structure of hepatic cells of experimental rats was also studied. The ethanol extract of *Annona reticulata* (EEAR) leaves possessed antioxidant activity as shown by significantly increased activities of scavenging enzymes, catalase (CAT), reduced glutathione (GSH) and superoxide dismutase (SOD) content as compared to CCl_4-treated rats in which the level of these enzymes were decreased. Administration of the extract also improved the lipid profile of the treated groups. Histopathology of the liver tissues showed that CCl_4 induces deterioration in cellular boundaries, the vacuolisation, inflammatory infiltration, dilation of sinusoidal, increased number of kupffer cells and the fatty degeneration (steatosis) in the liver. However, supplementation of leaf extract of *Annona reticulata* attenuated the cellular necrosis and led the CCl_4-induced alterations to restoration repairing of cells toward normal. These findings suggest that treatment of ethanol extract of *Annona reticulata* (EEAR) leaves exerts a therapeutic protective effect in CCl_4-treated rats by decreasing oxidative stress, and hepatic damage.

Key Words - *Annona reticulata,* CCl_4, CAT , GSH, SOD, LPO, Liver, and Rat

Introduction

Liver is the main organ of metabolism and excretion. It often bears load of a variety of xenobiotics and therapeutic agents. Drug-induced hepatic injury is one of the most common reasons alluded for withdrawal of an approved drug. Hepatic disease is a significant public health concern because it is reported to be the 11th leading cause of death and 15th leading cause of morbidity across the world (WHO, 2016). Over 900 drugs, toxins, and herbs have been reported and considered to cause liver injury, and drugs account for 20-40% of all cases of liver injury and its failure. The manifestations of drug-induced liver injury are highly unpredictable, varies from asymptomatic increase of liver enzymes to fulminant hepatic failure.

Most of the synthetic medicines during the treatment of many diseases like cancer, tumor and excessive alcohol consumption cause liver damage affecting adversely the health of human beings. While metabolizing many drugs, toxins and certain herbs liver gets affected and they become cause for liver diseases and hepatotoxicity. Similarly, neurodegenerative diseases, aging, inflammation, and acute and chronic liver diseases precipitate directly or indirectly through reactive oxygen species (ROS) mediated mechanisms (Liang *et al.*, 2011; Dutta, 2018) leading to oxidative stress in the liver. Oxidative stress on liver affects the antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), and glutathione reductase (GSH) and increase the lipid peroxidation (LPO) in liver Habib *et al.*, 2015). Antioxidants play important role to neutralize the free radicles produced in the body (Fu *et al.*, 2010).

Plants are natural source containing broad range of medicinally important compounds and currently public are more interested in herbal drugs as they are considered to be safer and cheaper as compared to synthetic drugs. Natural antioxidants of plants are responsible for preventing the damaging consequences of oxidative stress. Therefore, antioxidant based drug formulations are used for the prevention and treatment of variety of diseases. These compounds are free radical scavengers such as polyphenols, flavonoids and phenols **(**Khalaf *et al.,* 2008).

Till date, a proper curative therapeutic agent for liver disorder has not been found. Therefore, investigation of the usually concerned agent and a high index of suspicion are necessary in prognosis. Annona species has been reported to have potential antioxidative and hepatic protective properties (Vatakkee and Pramod, 2014, Haggag, 2016*)*. Ethanolic leaf extract of *A.reticulata* comprises effective potential source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses (Ahirwar and Tembhre, 2021). It has been used to treat various disorders such as epilepsy, cardiac problems, dysentery, worm infestations, bacterial infections, hemorrhage, dysuria, fever, and ulcers. The decoction of leaf of *Annona reticulata* is used as a vermifuge and its crushed leaves or a paste of the flesh is used as bandages on boils, swelling and ulcers for their treatment. The dried unripe fruit and bark decoction is employed against diarrhoea and dysentery (Bopana and Saxena, 2008).

Carbon tetrachloride (CCl_4) has been widely used in animal models to investigate chemical toxin-induced liver damage and hepatotoxicity in animal models (Manibusan, 2007; Wang, 2008). CCl_4 triggers lipid peroxidation in the liver during its metabolism into the highly reactive trichloromethyl radical (Basu, 2003). Therefore, blocking the lipid peroxidation can protect liver against CCl_4-induced injury. In this study, the hepatoprotective activity of the ethanol extract of *Annona reticulata* (EEAR) leaves was investigated on CCl_4-induced chronic liver injury in rat.

Materials and Methods

Plant material

The leaves of *Annona reticulata* were collected from local area of Bhopal, India, and were identified and authenticated by Dr. Zia Ul Hassan (H.O.D. Botany), Safia Science College, (Bhopal, Madhya Pradesh, India). The voucher specimens bearing numbers 508/Bot/Safia/14 and 509/Bot/Safia/14 were submitted in the said department for future references.

Preparation of plant extracts

About 300 gm of the leaf powder of *Annona reticulata* was extracted with 1.2 L of 90% ethanol using Soxhlet apparatus for 72 hrs at 40-50°C for seven days

Acute oral toxicity tests and dose selection

The acute oral toxicity study of the extracts of *Annona reticulata* was carried out according OECD (Organization for Economic Co-operation and Development) with the use of a minimum number of animals per step. Rats were administered orally at the doses of 5, 50, 300 and 2000 mg/kg b. wt and no mortality was observed. On the basis of acute toxic study, the dose of the extracts selected for the *in vivo* studies were 200 mg/kg there were no lethality observed up to 2000 mg/kg in the animals, hence, $1/10^{th}$ of that very dose was selected.

Test animals

Twenty four healthy male albino wistar rats weighing 120-150g were obtained from animal house of Pinnacle Biomedical Research Institute (PBRI), Bhopal Madhya Pradesh India. They were maintained in the polypropylene cages in controlled temperature 22 ± 2 ºC and light cycle (12 hours light and 12 hours dark) as per the guidelines of committee for the purpose of control and supervision on experiments on animals (CPCSEA). The animals were fed with the pellet diet and water *ad libitum.* Animal experiments were performed with prior permission from Institutional Animal Ethics Committee (IAEC) of PBRI, Bhopal (1283/C/09/CPCSEA). Body weight of the animals was recorded and were randomly divided into ten groups of six rats each and treated as follows for 30 days:

Experimental design

The animals were divided into four groups of six rats each.

- Group 1 served as normal control and received distilled water orally daily for 30 days.
- Group 2 served as toxic control and received $CC1_4$ (2 ml/kg body weight) weekly in olive oil (1:1, v/v, i.p.) for 30 days.
- Group 3 served as treatment group and received *Annona reticulata* ethanol leaf extract (200 mg/kg body weight) daily for 30 days.
- Group 4 served as treatment group and received $CC1_4$ (2 ml/kg body weight) weekly in olive oil (1:1, v/v) for 30 days and *Annona reticulata* ethanol leaf extract (200 mg/kg body weight) daily for 30 days.

The herbal formulation was suspended in distilled water and given orally through an intragastric tube daily in the morning.

Tissue Biochemical Assays

The animals were fasted overnight on the 30^{th} day. On the next day, the body weights of the animals were recorded and then euthanized with chloroform and

dissected thereafter. The liver was dissected out, blotted off blood and rinsed in freshly prepared ice cold saline. The fat was freed from the organ and a part of the liver was homogenized in phosphate buffer and certain portion in triss HCl buffer separately in Potter Elvehgen homogenizer fitted with polyteflon plunger at high speed. The homogenate, 10 % w/v thus obtained was centrifuged at 4500 rpm at 4ºC. The supernatant fraction thus obtained was analyzed for SOD (Paoletti *et al*., 1986), CAT (Goth, 1991), GSH (Ellman, 1959) and LPO, (Ohkawa *et al*., 1979).

Histopathology Examination

A portion of livers was collected in 10% buffered formaldehyde and preserved for at least 24 h. Further liver samples were dehydrated gradually with ethanol (70–100%), cleared in xylene and embedded in paraffin. Sections of 5 µm were prepared by microtome assembly and stained with hematoxylin and eosin to examine under microscope (40X) for histopathological alterations.

Statistical Analysis

All data were calculated as Mean±SD. Results were interpreted using One Way ANOVA followed by Benferroni's multiple comparisons test. $p<0.05$ and $p<0.001$ were considered as statistically significant.

Results

Effect of *Annona reticulata* Leaf Ethanol Extracts on Antioxidant Enzyme Levels

Effect of Ethanol Leaf Extract of *Annona reticulata* (EEAR) on the Levels of Superoxide Dismutase (SOD) in Experimental Groups of Rats

The level of SOD in control group of rats was 341.246±8.020 U/ml but the level of SOD was reduced to 212.061±12.930 U/ml highly significantly in the group treated with CCl_4. However, the group of rats supplied with 200 mg/kg of EEAR, the level of SOD was 334.373±7.535 U/ml, thus showing the non-toxic nature of *Annona reticulata* extract (Table - 1& Fig. 1). However, in the group of rats treated with the extract of *Annona reticulata* along with CCl_4, the level of SOD was highly significantly ($p<0.001$) restored to 296.491±16.761 U/ml.

Effect of Ethanolic Leaf Extract of *Annona reticulata* (EEAR) on the Levels of Catalase (CAT) in Experimental Groups of Rats

The level of CAT in control group of rats was 448.458±38.562 U/ml and CCl_4 intoxication decreased the level of CAT to 182.279±16.576 U/ml, ($p<0.001$). In only herb treated group, the level of CAT was somewhat near to control viz. 437.662±18.483 U/ml. However, in the group of rats treated with the extract of *Annona reticulata* alongside CCl_4, the level of CAT noticed was 294.202±14.315 U/ml which was significantly increased ($p<0.001$) as compared to CCl_4 intoxicated group of rats (Table-1 & Fig.1). These results clearly showed the protective nature of EEAR in combating the alterations in the levels of CAT upon CCl_4 intoxication.

Effect of Ethanolic Leaf Extract of *Annona reticulata* (EEAR) on the Levels of Glutathione (GSH) in Experimental Groups of Rats

In control group of rats, the level of GSH was 0.515±0.013 nM/g. However, in the group of treated with 2 ml/kg of CCl_4, the level was highly significantly ($p<0.001$) decreased to 0.293±0.025 nM/g and in the group of rats supplied with EEAR at 200 mg/kg, the level of GSH was confined to 0.506±0.039 nM/g (Table-1 & Fig.1). But the group of rats treated with EEAR+CCl_4 for the duration of 30 days, the level of GSH was significantly increased ($p<0.001$) to 0.377±0.015 nM/g, thus showing the efficacy of ethanol leaf extract of *Annona reticulata* in nullifying the negative effects of CCl_4.

Effect of Ethanol Leaf Extract of *Annona reticulata* (EEAR) on the Levels of Lipid Peroxidation (LPO) in Experimental Groups of Rats

In control group of rats, the level of LPO was 24.222±1.559 nM/ml while as in CCl_4 exposed group, the level was highly significantly ($p<0.001$) elevated to 53.333±3.989 nM/ml. However, the group of rats treated with the 200 mg/kg of EEAR, the level was 24.733±1.834 nM/ml and the group of rats treated with EEAR+CCl_4, the level of LPO was significantly decreased ($p<0.001$) to 33.028±2.182 nM/ml (Table-1 & Fig.1). These results clearly showed the protective nature of *Annona reticulata* leaf extract in combating the liver damage caused by carbon tetrachloride in rats.

Table: 1 – Effect of ethanol leaf extract of *Annona reticulata* (EEAR) on liver biochemical parameters for 30 days.

GROUPS	SOD (U/ml)	GSH (mM/gm)	CAT (U/ml)	LPO MDA (nM/ml)
Control	341.246±8.020	0.515±0.013	448.458±38.562	24.222±1.559
CCl_4	212.061±12.930* (-37.856%)	0.293±0.025* (-43.106%)	182.279±16.576* (-59.354%)	53.333±3.989* (+54.583%)
EEAR 200 mg/kg	334.373±7.535* (+36.579%)	0.506±0.039* (+42.094%)	437.662±18.483* (+58.351%)	24.733±1.834* (-53.625%)
EEAR 200 mg/kg+CCl_4	296.491±16.761* (+28.476%)	0.377±0.015* (+22.281%)	294.202±14.315* (+38.042%)	33.028±2.182* (-38.072%)

All data were represented in Mean ±SD, n = 6, * p < 0.001 compared to CCl_4 treated group. + = % increase and - = % decrease. CCl_4 group was compared with control and the rest of groups were compared with CCl_4 treated group.

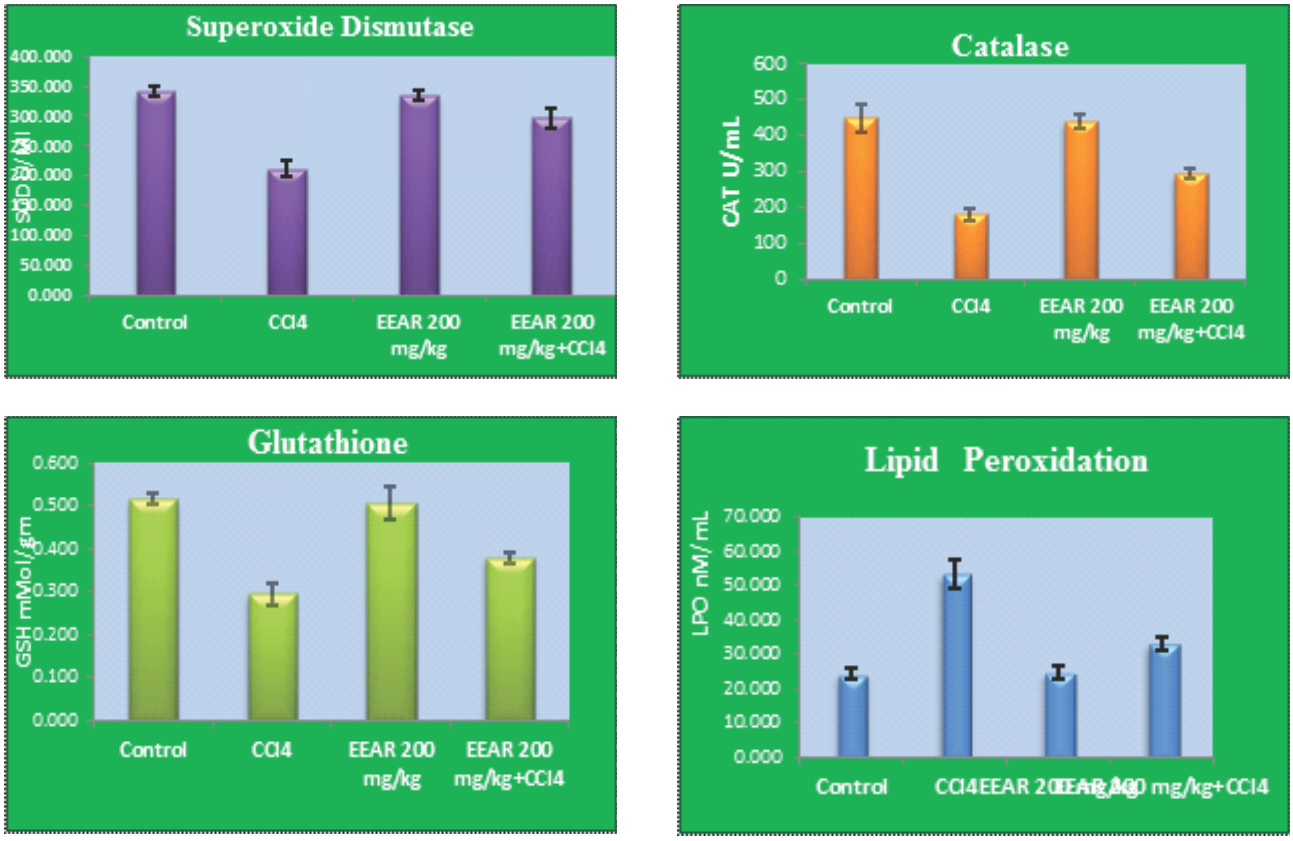

Fig. 1 – Showing the effect of ethanol leaf extract of *Annona reticulata* (200 mg/kg b.wt daily for 30 days) on the levels of antioxidant enzyme and lipid peroxidation (LPO) in rats intoxicated with CCl_4 for the duration of 30 days.

Result of Histopathology Studies

Liver histology was also carried out to confirm the potential of *Annona reticulata* leaves. The result found were quite satisfactory and in concurrence with those of biochemical results. The keen study of the liver sections of the rats belonging to the control group (group 1) displayed quit a normal cellular structure with well defined nucleated cells, sinusoids were prominent, central vein was also evident and portal triad (consisting of hepatic artery, bile duct and portal vein) was clearly visible (Fig. 2 – a & b).

However, in group 2 i.e. group treated with 2 ml/kg intraperitoneal dose of carbon tetrachloride with 50% olive oil weekly for 30 days, the cells lost their integrity with the loss of cellular boundaries, the vacuolisation was prominent, inflammatory infiltration was quit evident, dilation of sinusoidal spaces was also found, kupffer cells were in rich number and th e fatty degeneration (steatosis) was at peak (Fig. 3 a & b).

In group 3 i.e. animals supplied with 200 mg/kg herbal extract of *Annona reticulata* (Fig. 4. A & b) for 30 days, no changes in the cellular boundaries were found, the hepatocytes were with well defined nucleus, sinusoids were also clearly visible central vein was also found with normal structure. These findings clearly showed the non-toxic nature of the selected ethanolic extracts at the chosen doses.

However, in the group 4 i.e. the animals treated with 200 mg/kg *Annona reticulata* alongside CCl_4 for 30 days, the hepatocytes showed the restoration by retaining the cellular boundaries along with nucleus, reduction in the dimensions of sinusoids, meek steatosis and mild inflammatory infiltration (Fig. 5 a & b).

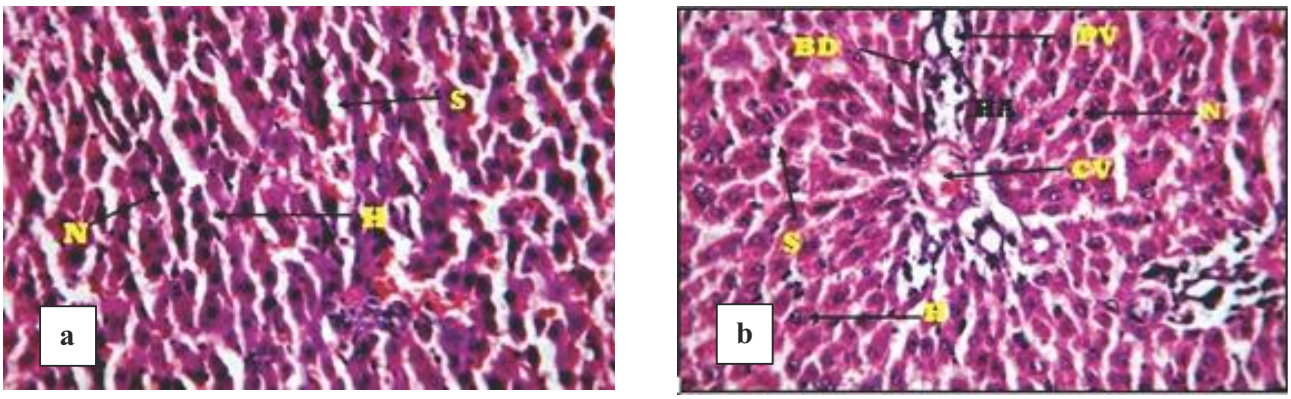

Fig. 2 (a) & (b) – Photomicrographs of liver sections of control group (1) of rats showing the normal hepatocytes (H) with well defined nucleus (N), central vein (CV), hepatic artery, bile duct, portal vein and regular sinusoids (S).

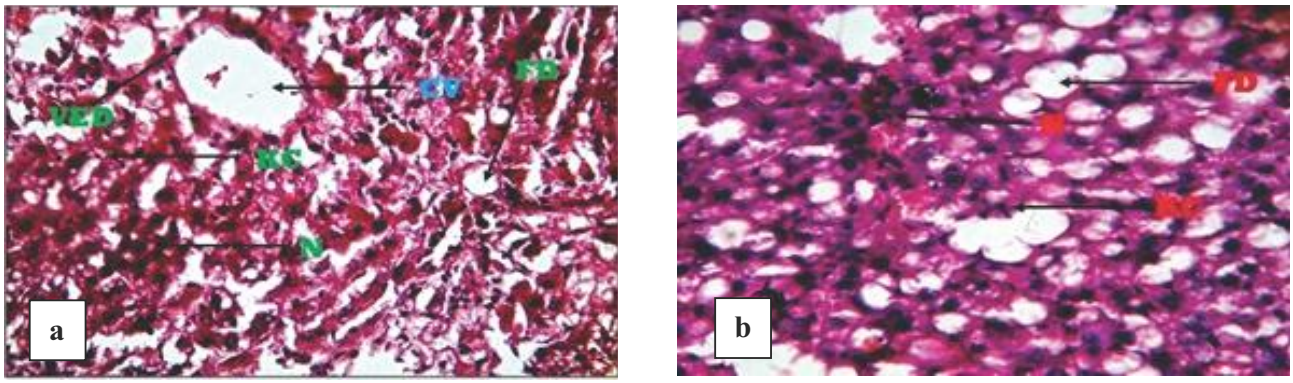

Fig. 3 (a) & (b) - Photomicrographs of liver of rats inebriated with CCl_4 (2 ml/kg weekly for one month), showing Kupffer cell (KC), Necrosis (N), Fat Degeneration (FD), Vascular Endothelial Damage (VED), Sinusoidal expansion (SE), Portal Vein (PV), Bile Duct (BD) and Hepatic Artery (HA) (40X, haematoxylin-eosin stain).

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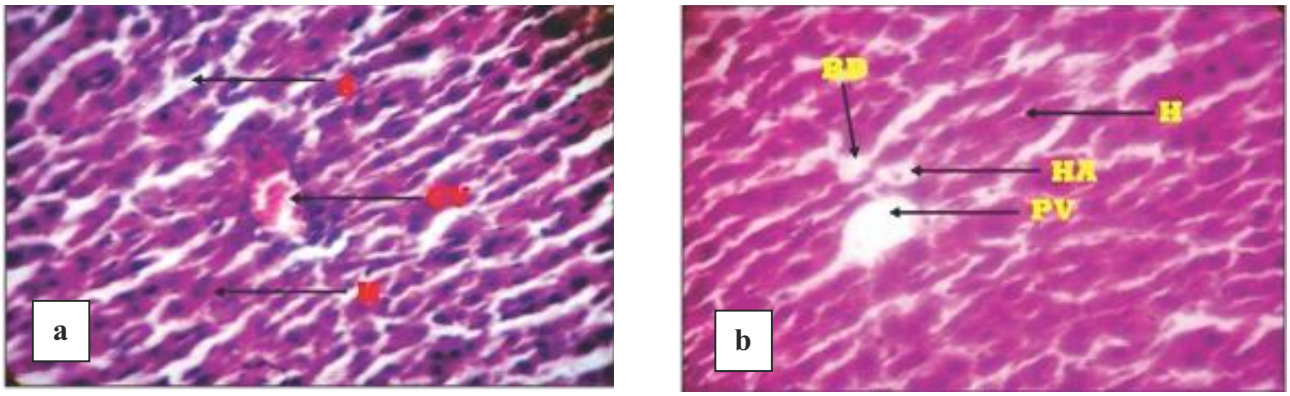

Fig. 4 (a) & (b)- Photomicrographs of liver of rats supplied with ethanolic extract of *Annona reticulata* showing well arranged hepatocytes with prominent nucleus, normal sinusoids, central vein, Portal Vein (PV), Bile Duct (BD) and Hepatic Artery (HA) (40X, haematoxylin-eosin stain).

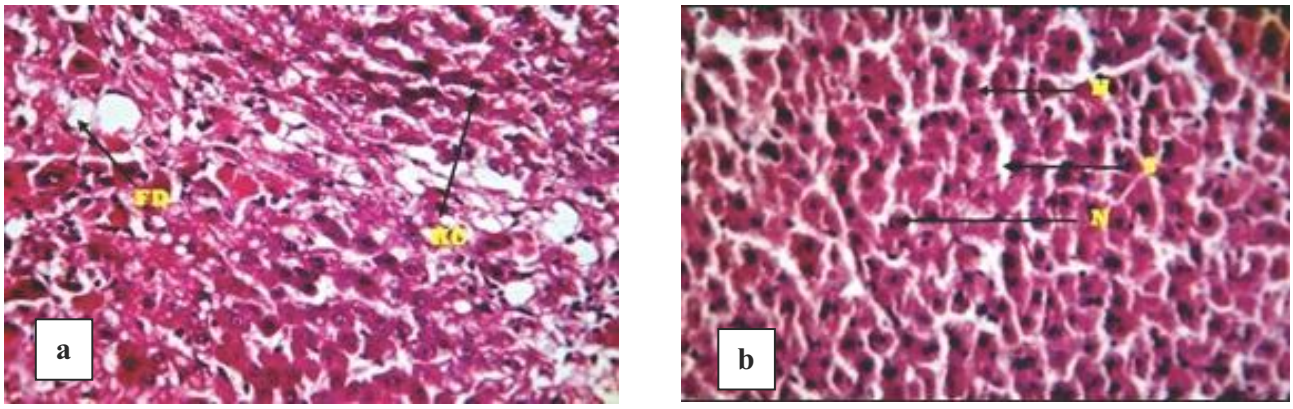

Fig. 5. (a) & (b) – Photomicrographs of liver sections of rats treated with *Annona reticulata* 200 mg/kg along with weekly dose of CCl_4 showing the normal hepatocytes (H) with well defined nucleus (N), central vein (CV) and regular sinusoids (S).

Discussion

The extensive survey of the available literature depicts that free radicals are usually produced during the normal metabolic pathways though certain can be acquired from the environment also. Free radicals are the species which contain an unpaired electron or electrons. The normal physiological pathways frequently generate oxygen radicals, such as superoxide radical (O^{2-}), hydroxyl radical (OH) and non-free radical species, such as singlet oxygen (O_2) and hydrogen peroxide (H_2O_2) (Gulcin *et al.,* 2002). But the nature has provided the animals with a wonderful system to cope up with the free radicals known as antioxidant defence system, which consists of various enzymes such as superoxide dismutase, catalase and glutathione peroxidise etc. The deficiency of vitamins along with excessive production of free radicals and a diminished level of above captioned enzymes is regarded as the main cause for imposing oxidative stress (Ellnain-Wojtaszek *et al*., 2003). SOD has been reported as one of the most important enzymes in the enzymatic antioxidant defence system. It scavenges the superoxide anion to form hydrogen peroxide (Margaill, 2005). The H_2O_2 thus formed becomes the site for catalase to act upon and is finally broken down into water and oxygen molecule. SOD also maintains the normal atmosphere of the body by neutralizing the free radicals as well as takes part in the detoxifying reactions (Pushpakiran *et al*., 2004).

Catalase is the important antioxidant enzyme found in all animal tissues though its maximum activity is found in liver and red blood cells. As already mentioned, catalase crumbles hydrogen peroxide into water and oxygen molecule, thereby protects the cells from the dangerous hydroxyl radicals (Chance *et al.,* 1952).

Glutathione is one of the most profuse tripeptide, a non-enzymatic biological antioxidant found in the liver. The prime functions of glutathione are to remove wandering free radicals and maintenance of thiol proteins. It also acts as the substrate for

glutathione peroxidase and GST (Prakash *et al.,* 2001).

The level of lipid peroxide is a measure of membrane damage and alteration in structure and function of cellular membranes. Elevation of MDA level in liver indicates excessive free radical generation and consequently enhanced lipid peroxidation which leads to severe tissue damage (Shenoy *et al.,* 2001). The elevated thiobarbituric acid reactive substances (TBARS) level suggests enhanced lipid peroxidation which finally leads to tissue damage and malfunction of natural antioxidant defense mechanisms to avert formation of excessive free radicals (Szymonik *et al.,* 2003).

In the present study, we analyzed the *in vivo* antioxidant nature of *Annona reticulate* leaves*, on the* levels of superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and lipid peroxidation (LPO) in different groups of rats.

In control group of rats, the level of SOD observed was 341.246±8.020 U/ml, while as the highly significant depression in the level of SOD by -37.856% was noticed in the group of rats subjected to carbon tetrachloride intoxication. Supplying the rats with 200 mg/kg ethanolic extract of *Annona reticulata* (EEAR) has not shown any noteworthy change in the level of SOD as evident by its level 334.373±7.535 U/ml (close to that of control). However, in the test group of rats i.e. treated with 200 mg/kg of EEAR and concurrently subjected to carbon tetrachloride, the level was highly significantly increased by +28.47% as compared to that of only carbon tetrachloride treated group.

As for as the level of GSH is concerned, it was 0.506±0.013 mM/gm but CCl_4 exposure decreased it by -43.106% ($p<0.001$). In the group of rats supplied with the EEAR at 200 mg/kg, the level of GSH was almost similar to that of control group viz. 0.501±0.031 mM/gm. On the other hand, a highly significant protection was noticed in the group of rats supplied with EEAR 200 mg/kg+CCl_4 as apparent from the increased level of GSH by +22.281.804 as that of the only CCl_4 treated group.

The level of CAT in control group of rats was 448.458±38.562 U/ml however; the level was highly significantly decreased by -59.354% upon CCl_4 intoxication. No significant change was noticed in its level in the group of rats supplied with 200 mg/kg of the EEAR as obvious from its level viz. 437.662±18.483 U/ml. However, the group supplied with 200 mg/kg of EEAR and simultaneously intoxicated with CCl_4, showed the marked protection by increasing the level of GSH by +38.042% as compared to that of only CCl_4treated group.

The control level of LPO noticed in the present study was 24.222±1.559 nM/ml but in CCl_4 treated group, the level was highly significantly elevated by +54.583%. On the other hand, the group treated with only EEAR by 200 mg/kg showed the level of LPO similar to that of control group viz. 24.733±1.834 nM/ml. However, the mixed group i.e. treated with EEAR at 200 mg/kg and concomitantly inebriated with CCl_4 showed the highly significant protection by decreasing the level of LPO by -38.072%.

In an experiment Fakurazi *et al.* (2008) reported the ethanol leaf extract of *Moringa oleifera* offers protection to level of glutathione in rat liver injury induced by acetaminophen. the significant protective nature of the extract was confirmed when the level of the glutathione was elevated to 2.59±1.60 µmol/g in the group treated with both the extract and acetaminophen. The ethanolic extract of leaves of *Phyllanthus polyphyllus* at 200 mg/kg for ten days restored the levels of LPO, SOD and catalase to 7.51±1.38 µ moles of MDA/ min/mg protein, 23.48±1.04 Units/min/mg protein and 45.17±2.10 µ mole of H_2O_2 consumed/ min/mg protein respectively in rats treated with CCl_4 (Rajkapoor *et al.,* 2008). Kumar *et al.* (2010) while evaluating the antioxidant effect of methanolic leaf extract of *Caesalpinia bonducella* in rats challenged with CCl_4 found the normal levels of LPO (n mole of MDA/mg protein), GSH (µg/mg protein), SOD (U/mg protein) and catalase (U/mg protein) as 0.92±0.05, 5.45±0.29, 93.36±5.35 and 354.61±15.07 respectively. The highly significant alteration was noticed in the levels of LPO (n mole of MDA/mg protein), GSH (µg/mg protein), SOD (U/mg protein) and catalase (U/mg protein) upon CCl_4 exposure viz. 7.45±0.31, 0.67±0.32, 52.23±2.26 and 267.92±12.07 respectively. The protection was offered by the methanolic leaf extract of *Caesalpinia bonducella* at 50 mg/kg in rats challenged with CCl_4as specified by the levels of LPO (n mole of MDA/mg protein), GSH (µg/mg protein), SOD (U/mg protein) and catalase (U/mg protein) viz. 4.26±0.21, 1.29±0.06, 59.12±2.23 and 281.14±15.03 respectively.

Singh *et al.* (2010) carried out the similar sort of work as that of ours and found the basic levels of SOD (U/ mg of protein), catalase (U/ mg of protein) and lipid peroxidation (nm MDA/g of protein) in rats as 12.75±0.55, 178±28.8 and 6.12±0.87 respectively. The significant alteration was found in the levels of SOD (U/ mg of protein), catalase (U/ mg of protein), peroxidase (U/ mg of protein) and lipid peroxidation

(nm MDA/g of protein) viz. 7.44±0.14, 75.56±0.67, 112±8.88 and 24.76±2.56 upon CCl_4 (0.25 ml/100 gm, once in week) inebriation. Kowti *et al*. (2013) witnessed the significant amelioration by the ethanol extract of *Mentha arvensis* leaves in rats exposed to CCl_4 at the 400 mg/kg dose of the extract with the levels of in the levels of GSH, SOD, CAT and MDA.

The histopathological studies are direct evidence of efficacy of drug as protectant. In the present study, the protective efficacy of *Annona reticulata* (leaves) was evaluated in the rats subjected to CCl_4 exposure. The extracts were supplied to the rats daily at 200 mg/kg and CCl_4 (2 ml/kg) was injected once in a week for 30 days. The experimental groups were compared with the control group and the effect of the herbal extracts was determined.

The liver sections of control group of rats comprised of well bordered hepatocytes with clear nuclei, sinusoids were definite and clearly visible, structures like bile duct, hepatic artery and portal vein were of usual shapes. But, the exposure of carbon tetrachloride (2ml/kg, once a week for 30 days) produced the significant changes with loss of hepatic borders, widening of sinusoids, inflammatory infiltration, fatty degeneration (steatosis) and increased kupffer cells. However, in the group of rats treated with ethanol extract of *Annona reticulata* leaves (200 mg/kg) has not shown any significant changes in the liver sections, thus showing the non toxic effects of the said herbs on liver. The group treated with ethanolic extract of *Annona reticulata* leaves at 200 mg/kg+CCl_4, showed the almost similar structure as that of control group but here in this group, the kupffer cells were more in number and vacuoles were still seen. The outcome of the above histopathological studies clearly showed that *Annona reticulata* leaves offered protection. These results were in strong agreement with the results of our own biochemical studies.

Ahsan *et al.* (2009) scrutinized the hepatoprotective effect of methanol extracts of *Casuarina equisetifolia* (Leaf and bark), *Cajanus cajan* (whole plant), *Glycosmis pentaphylla* (leaf and bark), *Bixa orellana* (seed), *Argemone mexicana* (leaf and flower), *Physalis minima* (whole plant), *Caesalpinia bonduc* (leaf and bark) in rats treated with CCl_4. CCl_4 (3ml/kg) intoxication caused disarrangement of hepatic cells with centrilobular hepatic necrosis, sinusoidal expansion, kupffer cell hyperplasia, central vein crowding and apoptosis. Supplying the rats with the extracts of four plants, *Casuarina equisetifolia*, *Cajanus cajan*, *Glycosmis pentaphylla*, *Bixa orellana* at a dose of 250 mg/kg and 500 mg/kg b.wt. showed reasonable to weak activity in protecting the liver cells from CCl_4 injury. Amongst these plant extracts, *Bixa orellana* extract almost returned the hepatic architecture to normal.Similar results were noticed with diethyl ether extract of leaves of *Coccinia indica* (Kumar *et al.*, 2010) and aqueous extract C*rossostephium chinensis* (Chang *et al.,*2011) in rats treated with CCl_4. The cellular alterations like steatosis, perivenular fibrosis and inflammatory infiltrations were common in CCl_4 exposed group. The concurrent treatment of the CCl_4 exposed rats with methanolic leaf extract of *Mallotus Philippensis* at two selected doses of 100 mg/kg and 200 mg/kg showed less hepatocellular damage as compared to that of only CCl_4 treated group thereby indicating the hepatoprotective activity of the selected medicinal plant (Ramakrishna , 2010).

Hemamalini and Sathya (2013) carried out the work focused on histological investigation of liver of rats treated with CCl_4 (1.5 ml/kg) and methanolic extracts of *Sophora interrupta* and *Holoptelea integrefolia.* The investigators found that carbon tetrachloride significantly caused the mass changes in the histology of the liver like perivenular fibrosis, fatty changes in hepatocytes and inflammatory infiltrations. However, supplying the groups of rats with the methanolic leaf extract of *Sophora interrupta* (400 mg/kg) and *Holoptelea integrefolia* (500 mg/kg) daily for seven days prevented the changes caused by CCl_4 thereby showing the protective nature of both the herbs.

Conclusion

The results of the biochemical studies revealed that extract of *Annona reticulata* leaves (200 mg/kg) showed decent hepatoprotective activities. The *in vivo* antioxidant efficacy of the extracts was also established by analysing the levels of Superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and lipid peroxidase (LPO) in the liver homogenate of different groups of rats and the results obtained depicted that the significant protection was displayed by *Annona reticulata* leaves. In addition to the biochemical studies, the present study was stretched to analyse the histopathological changes in different secs of rats as well. The results of the histopathological studies were in full agreement with those of our biochemical studies.

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