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"Evaluation of hepatoprotective and antioxidant activity of *Tephrosia purpurea* L. against carbon tetrachloride intoxicated wistar rats"

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Abstract
Present investigation was carried out to study antioxidant activity of aqueous extract of *Tephrosia purpurea* fruit against carbon tetrachloride intoxicated Wistar rats. Forty-eight rats of either sex randomly divided into six groups, 8 rats (4 male and 4 female) in each group. Group I as healthy control, Group II was given CCl_4, @ 0.2 ml (1 ml in 1 ml liq. Paraffin) i/p daily for 28 days as toxic control while group III administered with silymarin @25 mg/kg, p.o. served as positive control, the group IV, V and VI were given aqueous fruit extract of *Tephrosia purpurea* @ 50, 75 and 150 mg/mg body weight respectively for 28 days as the treatment groups. The antioxidant, parameters were assessed at 28th day of treatment.
There was significant improvement in antioxidant parameters of LPO, SOD, and CAT against CCl_4 hepatotoxicity in rats; these parameters were significantly reverse by AETP (III to VI) treatment in dose and duration dependent manner. The higher dose of extract treatment in group VI (150 mg/kg) on 28th day was most effective than other levels of treatment whose effect was comparable with silymarin (group III). Also pathology of liver was studied at the end of experiment.

Keywords: LPO, SOD, CAT, Histopathology Liver

Introduction
Antioxidants are the exogenous or endogenous compounds that prevents/intercepts/inactivates generation of free radicals, toxic reactive oxidants by blocking chain reactions [8]. They act either by breaking the oxidative chain-reaction or by removing ROS by quenching chain-initiating catalysts their biological effects are due to different mechanism (electron donation, metal ion chelation, co antioxidants or by gene expression regulation). Enzymatic antioxidants react with oxidizing chemicals or reduced their ability to cause detrimental effects [11]. are superoxide dismutase, glutathione peroxidase (GPx) and CAT, where superoxide dismutases evolve in breakdown of superoxide anion into oxygen and hydrogen peroxides, GPx, (selenium containing antioxidant) scavenges hydroperoxides radicals (product of lipid oxidation). The non enzymatic antioxidants like glutathione, ubiquinol and uric acid are second line defense produced by normal metabolism within the body, other like carotenoids, vitamin C and vitamin E are present in foods like soybeans, green tea, red wine and citrus fruits are of vegetable origin contains mostly the phenolic and polyphenolic compounds possesses the antioxidant potential.
Use of medicinal plants for the treatment of various diseases has been part of human culture since ancient times. Even with scientific advancement there is no specific remedy have been found to cure hepatic diseases, especially for chronic liver conditions in man and animals, therefore it is imperative to search new remedy from herbal sources. *Tephrosia purpurea* is commonly known as "Sharapunkha" in Sanskrit, is a traditional herb was used in treatment of bilious febrile attacks and in liver and spleen obstructions have possess deodorant, tonic, diuretic, laxative properties and useful in bilious febrile attack, cough, lightness of chest, biliary and splenic troubles and liver diseases [3, 10, 14]. Currently, "Tephroli" and "Yakrifit" a polyherbal formulations contains *Tephrosia purpurea* are recommended for liver disorders [2] Some studies showed hepatoprotective activity of *Tephrosia purpurea* in rat [7, 9], while there is no report on hepatotoxicity evaluation of whole fruit (corticated) of *Tephrosia purpurea* in rodents, the present study evaluated the hepatoprotective and antioxidant activity of whole fruit (corticated) aqueous extract of *Tephrosia purpurea* against CCl_4 induced hepatic injury in wistar rats.

Material and Methods

Collection and Authentication of Plant

Whole fruits of *Tephrosia purpurea* were collected from waste land and cultivated agriculture land of local area and authenticates from the expert Botanist, Department of Botany, VNMKV, Parbhani. Fruits of *Tephrosia purpurea* were handpicked, collected was shade driedand ground into coarser powder by using electrical grinder.

Preparation of Aqueous Extract

The fruit powder of *Tephrosia purpurea* was subjected to cold maceration to obtain aqueous extract. 50 grams of fruit powder was dissolved in 500 ml distilled water mixed thoroughly was macerated at 4^0C for 48 hours. The mixture was intermittently shaken, after complete maceration, it was filtrate first through muslin cloth, then by ordinary filter paper, the filtrate thus obtain was transfer on glass Petri plates and allow to air dry, the residue left (extract) was collected in Petri plates and stored in air tight desiccators and used when required in this study.

Experimental protocols: The study was carried out in Laboratory Animal House, Department of Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Parbhani. M.A.F.S.U., Maharashtra. study were conducted on Forty-eight wistar rats of 5 weeks old, weighing 150-200 g of either sex were divided into six equal groups were employed in this study. Each group comprised of 8 rats (4 male and 4 females) of. Animals were selected after physical and behavioral examinations. At the time of randomization, the live body weights of rats were ranged within ±10 percent of the mean body weight. These animals housed in polypropylene cages(Four rats / cage) were maintain as per the CPCSEA guidelines on standard laboratory conditions was provided *ad-libitum* food and water. The approval of the institutional animal ethical committee was obtained prior to commencement of the experiment. After 5 days of acclimatization the animals were assigned to experimental group. Group I served as healthy control, Group II was given CCl_4, @ 0.2 ml (1 ml in 1 ml liq. Paraffin) i/p daily for 28 days kept as toxic control while group III administered with silymarin @25 mg/kg, p.o. served as positive control, the group IV, V and VI were given aqueous fruit extract of *Tephrosia purpurea* @ 50, 75 and 150 mg/mg body weight doses respectively for 28 days were the treatment groups.

Procedure

Preparation of liver tissue homogenate

After scarifying the rats liver was collected, washed thrice with the normal saline, then it was transferred to PBS (pH 7.4) and adjusts the volume of 1 liter with distilled water. It was stored in deep freeze and maintained at-20^0c temperature. Frozen liver samples before used it was partially thawed and prepared 10% (w/v) of liver tissue homogenate. 200 mg of liver sample was weighed and taken in 2 ml of ice-cold saline was triturated by using IKA homogenizer (Germany,) under ice-cold condition, then centrifuged at 3000 rpm for 10 minutes. The supernatant was used for estimations of the antioxidants.

Lipid peroxidation was measured in terms of malondialdehyde production according to the method of Thiobarbituric acid (TBA) described by Rehman [17].

The Catalase activity estimated by the method of Bergmeyer using diluted (1:10) liver haemolysates. [1] Superoxide dismutase activity was estimated as per the procedure described by Madesh and Balasubramanian. [12].

Table 1: Antioxidants of liver tissue in six different groups of wistar rats treated with AETP as compared to control

Groups	Antioxidant Parameters		
	MDA (LPO) (nM /ml)	CAT (µM /min/mg)	SOD (U/mg)
I	22.91±0.34^{e}	64.43±2.45^{a}	43.22±1.72^{a}
II	40.85±1.75^{a}	44.86±0.86^{d}	28.52±1.84^{c}
III	26.36±0.89^{d}	58.13±2.09^{b}	40.85±2.36^{ab}
IV	35.05±0.73^{b}	47.56±0.81^{cd}	35.67±3.43^{b}
V	32.12±0.71^{c}	50.67±2.83^{c}	38.77±2.24^{ab}
VI	22.82±0.33^{e}	56.07±0.84^{b}	42.22±1.48^{a}
Stat	2.46	5.28	6.49
CD	S	S	S

Statistical analysis: quantitative data were analyzed using CRD.

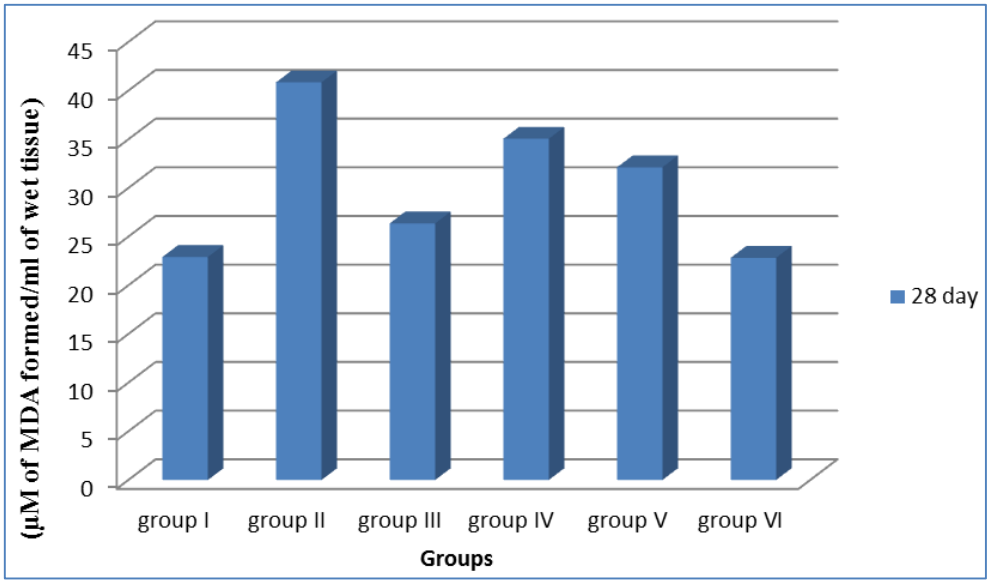


Fig 1: Malondialdehyde (MDA) concentration in liver tissue in different groups of wistar rats after AETP treatment

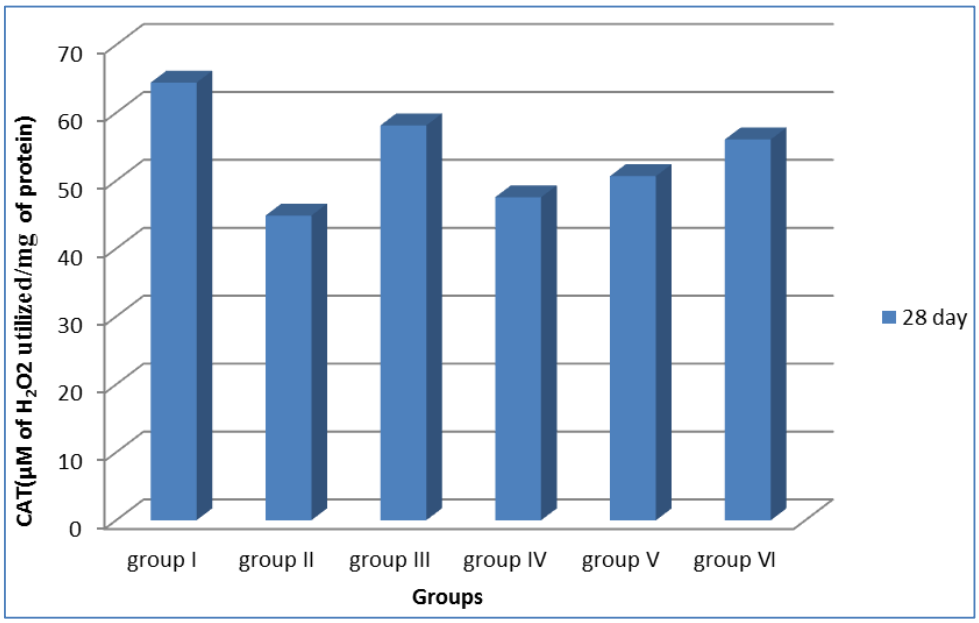


Fig 2: Catalase activity in liver tissue in different groups of wistar rats after AETP treatment

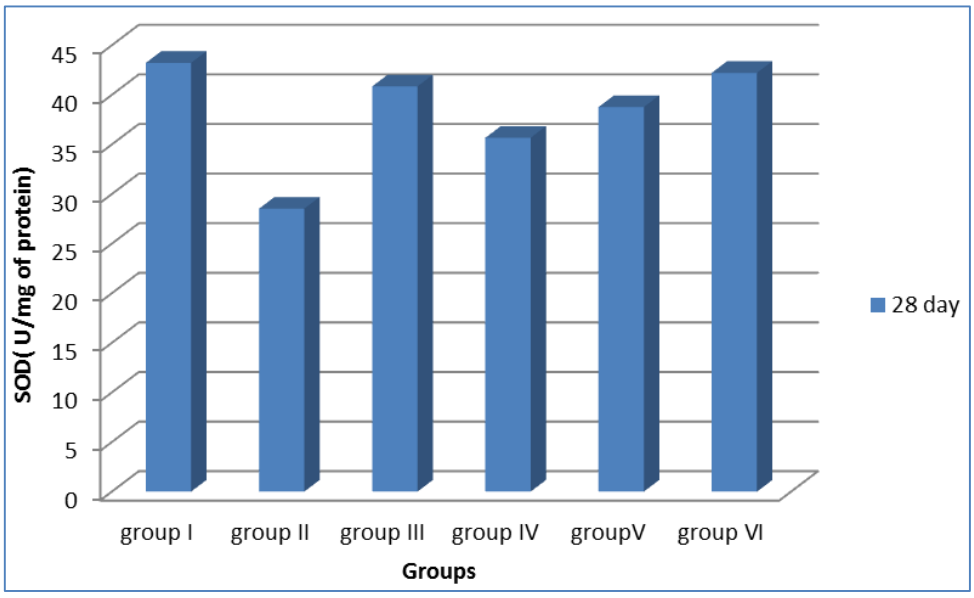


Fig 3: Superoxide dismutase in liver tissue in different groups of wistar rats after AETP treatment.

Results

The mean values of MDA concentration in liver tissue homogenates in group I, II, III, IV, V and VI were 22.91±0.34, 40.85±1.75, 26.36±0.89, 35.05±0.73, 32.12±0.71 and 22.82±0.33 respectively on 28th day of experiment. There was significant increase in MDA concentration in group II (toxic control), the increase level of MDA due to oxidative stress caused by CCl4 is reported in several studies [6, 13, 15, 21]. Significantly differs from the group IV, V and VI than group III observed compared to control (group I). The calculated MDA values in descending order of efficacy were in group II, IV > V > III > VI ≈ I which indicate CCl4 intoxication (group II) in rats shows highest MDA values followed by AETP-50 (group VI), AETP-75 (group V), Silymarin (group III), AETP-150 was the lowest value than untreated control (group I). There was significantly reduced MDA concentration in treatment group IV (35.05±0.73) and V (32.12±0.71) as compared to group II (40.85±1.75), which was comparatively lower than group III (26.36±0.89) when compared to group I (22.91±0.34) and significant decrease MDA level observed in group VI compare to group I [9]

The CAT activity in liver tissue homogenates of group I, II, III, IV, V and VI were 64.43±2.45, 44.86±0.86, 58.13±2.09, 47.56±0.81, 50.67±2.83 and 56.07±0.84 mM of H_2O_2 utilized/min/mg of protein respectively. There was significant decline in CAT activity in liver of group II (toxic control) might be due to oxidative stress induced hepatic damage by CCl4 in wistar rats. In groups IV, V and VI treatment of AETP significantly increase CAT activity in dose dependent manner, where the highest in group VI showed comparable CAT activity with the silymarin (group III). Accordingly the CAT activity graded in descending order as 58.13 (III) > 56.07 (VI) > 50.67 (V) > 47.56 (IV) > 44.86±0.86 (II) against 64.43 control (group I), where highest CAT activity was observed with silymarin (Group III) and the lowest with CCl4 toxic control (group II). This indicates that the CCl4 leads to hepatic damage (group II) resulting in significant reduction in CAT activity was improved by treatments with extract not differs from silymarin treatment (group III), however not restored to group I (control) level (64.43±2.45). This implies

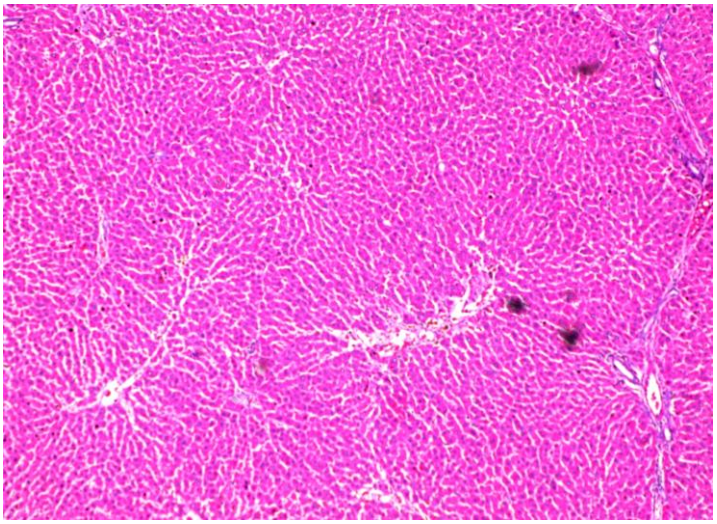
that AETP treatment in group IV, V and VI were significantly increase the CAT activity than group II (Toxic control) when compared to control (group I).

The mean values of SOD in group II (28.52±1.84), III (40.85±2.36), IV (35.67±3.43), V (38.77±2.24) and VI (42.22±1.48) units/mg of protein respectively against control (group) I (43.22±1.72). There was significant decrease in SOD activity in group II (28.52±1.84) when compared to control (43.22±1.72) might be due to CCl4 induced oxidative stress in this group. SOD activity in group III, IV, V and VI were significantly increased than group II and lower than control (group I). There was no significant difference observed in group III, IV and V when compared with group I and SOD value in group VI not significantly differs than group III and V when compare to control.

Histopathological alterations

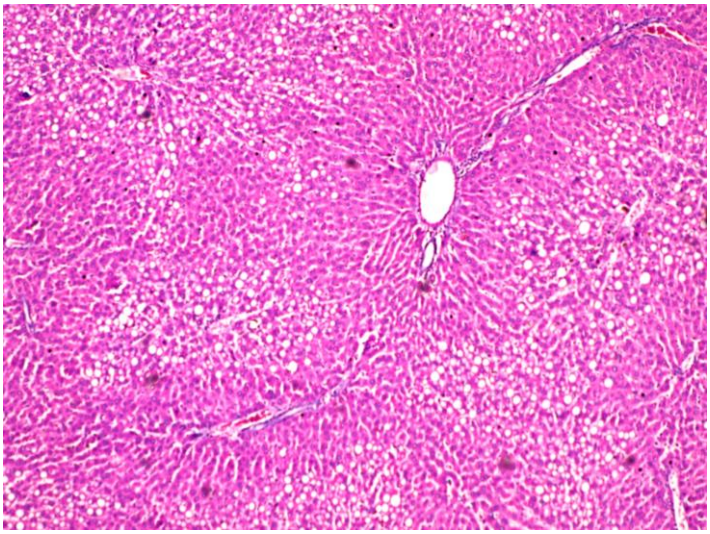
During experimental evaluation of the hepatoprotective and antioxidant activity of aqueous extract of *Tephrosia purpurea* against carbon tetrachloride induced hepatotoxicity in wistar rats, the histopathological examination of liver tissue were attempted at the end of trials (on 28th day).

Histopathological examination of liver tissues of control group did not revealed any histoarchitecture changes.



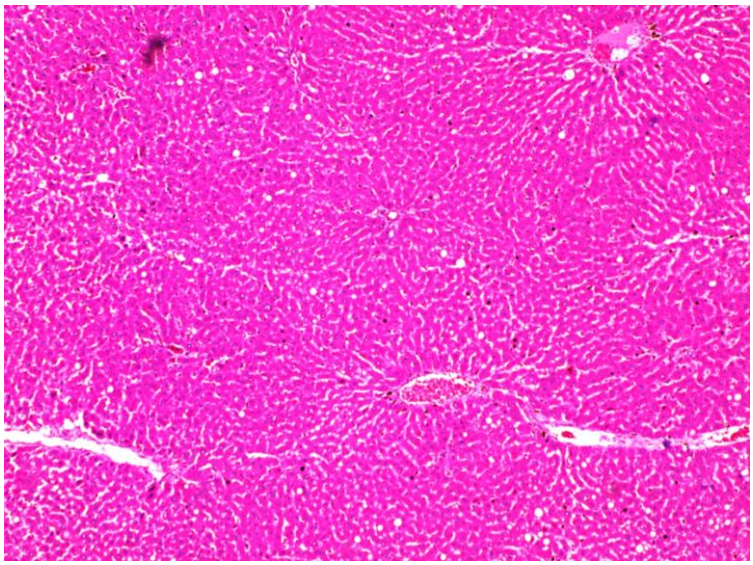
Microphotograph of liver within normal histological Limits from group I (control) (H and E ×100)

The sections of liver from the rats of group II intoxicated with CCl_4 daily for 28 days revealed mild to severe, focal to diffused fatty changes, necrotic changes, congestion, central vein dilatation and mononuclear cell infiltration.

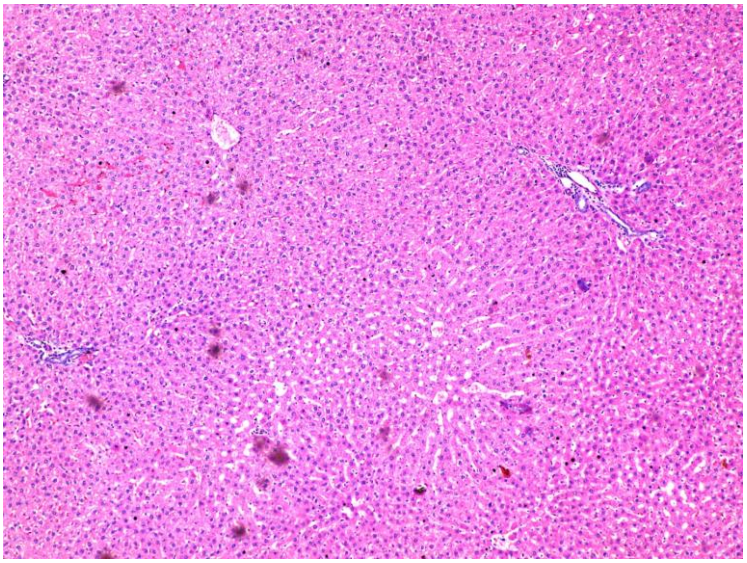


Section of a liver showing multifocal fatty changes and Necrosis in rat of group II (Toxic control) (H and E ×100)

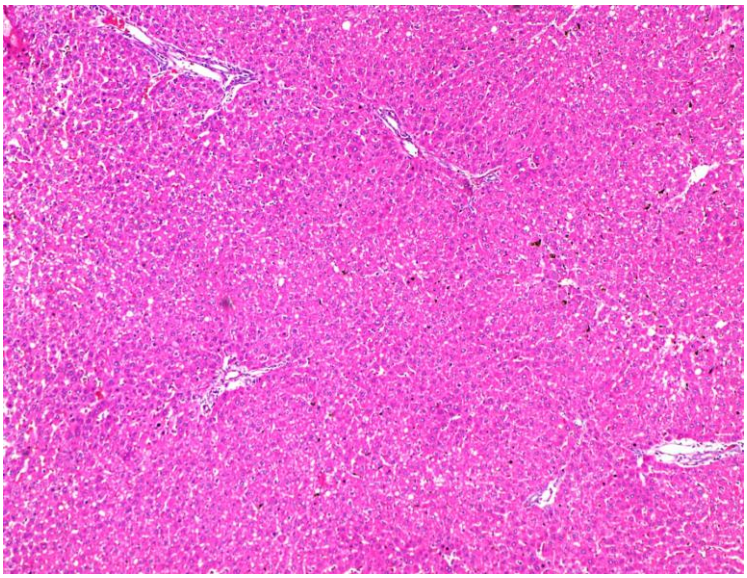
The sections of liver from the rats of group III and VI showed minimal fatty and degenerative changes were comparatively less intense than in rats of group II indicating beneficial effect of extract treatment at higher doses. However, the histopathology of liver from the rats of group IV and V showed fatty change, mononuclear cell infiltration, degenerative and necrotic changes at places.



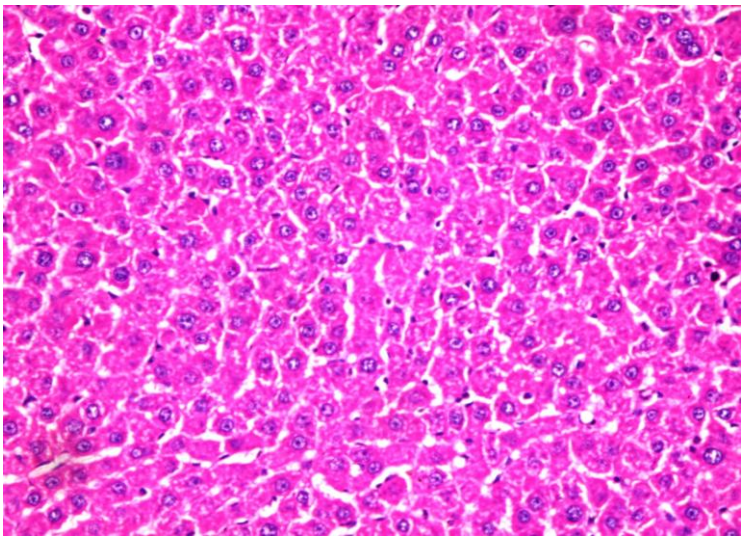
Congestion and minimal focal fatty change in hepatic Parenchyma of a rat from group III (silymarin) (H and E ×100)



Section of a liver from a rat of group IV showing fatty Changes and multifocal mononuclear cell infiltration (H and E ×100)



Eosinophilic degenerative changes, fatty changes and Mononuclear cell infiltration in hepatic parenchyma of Rat from group V (H and E ×100)



Microphotograph of liver with minimal focal fatty change from a rat of group VI (H and E ×100)

After treatment with AETP macroscopically liver tissue showed improvement in histoarchitecture with minimal fatty changes and degenerative changes, mononuclear cell infiltration and necrotic changes at place [7, 5, 19].

Discussion

Several studies showed significant reduction in SOD and CAT activity and increase LPO levels in liver tissue due to CCl4 intoxication in rats. [4, 13, 20] Our findings are accordance with these reports showed significant reduction in SOD and CAT in rats of group II treated with CCl4. Overall, CCl_4 induced hepatotoxicity increases MDA level, reduced CAT and SOD activity in liver tissue leads to the formation of excessive free radicals and tissue damage due to failure of antioxidant defense mechanism [13] inactivation of reactive oxygen species increases levels of SOD and CAT against CCl_4 induced hepatotoxicity in rats. [16, 18]

The increase liver antioxidants including lipid peroxidation (MDA activity), Catalase (CAT) and superoxide dismutase (SOD) against CCl4 intoxication in rats (group II) were significantly alter and reverse to normal among treatment group (III. IV, V and VI) as compared to control (group I). The increase lipid peroxidation (MDA activity) and reduced CAT and SOD highly significant in group VI on 28th day of treatment than 7th and 14th. This suggests CCl4 induced oxidative stress due to free radicals formation was significantly neutralized by enzymatic antioxidants and improved significantly antioxidant status in group VI (150 mg/kg) on 28th day of treatment with AETP was comparable with silymarin (III).

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