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Hepatoprotective Activity of Leaves of *Parkinsonia Aculeata* Linn Against Carbon -Tetrachloride Induced Hepatotoxicity in Rats.

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ABSTRACT

Liver is a vital organ performing wide range of functions ,oxidative damage is implicated in the pathogenesis of various liver disorders. Present study was aimed at evaluating protective ability of *Parkinsonia aculeata* Linn against CCl_4 -induced hepatotoxicity in rats .Seven experimental groups of six rats each were made.Petroleum ether , methanolic ,chloroform,and aqueous leaves extract were used for evaluation of hepatoprotective activity administered orally in a daily dose of 500 mg/kg for 10 days. In vivo hepatoprotective activity was assessed by measuring biochemical parameters like various enzymes,triglycerides and thiopental induced sleeping time potentiation. Results were also confirmed histopathologically. Pet ether and methanol extracts showed the strongest hepatoprotective effect comparable with standard drug Liv 52.No signs of toxicity was observed at 500 mg/kg daily dose.The hepatoprotective effects of *P. aculeata* may be due to both the inhibition of lipid peroxidation and the increase of antioxidant activity.

Keywords: *Parkinsonia aculeata*, Carbon tetrachloride (CCl_4), Hepatotoxicity, Thiopental.

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INTRODUCTION

Liver is the major organ responsible for metabolism of many synthetic chemical substances/drugs thereby providing protection against foreign substances by detoxifying and eliminating them[9]. This may lead to generation of free radicals causing hepatotoxicity by damaging the cell membrane and cell constituents[1]. Human beings have inbuilt natural endogenous antioxidant defence system against this oxidative stress by scavenging the generated free radicals. The inbuilt scavenging systems are the Glutathione (GSH), Superoxide dismutase (SOD) and Catalase (CAT)[1]. But prolonged production of large amount of free radicals. There is increasing evidence that free radicals and reactive oxygen species play a crucial role in various steps that initiate and regulate the progression of liver diseases independent of the agent of origin. By virtue of its unique vascular and metabolic features, the liver is exposed to absorbed drugs and xenobiotics in concentrated form [7]. Thus many drugs have greater tendency to cause hepatotoxicity as well other organ toxicities. Flavonoids & other phenolic compounds of plant origin have roles as scavengers and inhibitors of lipid peroxidation. CCl_4 whose catabolism produces radicals is commonly used for induction of liver damage in rats. The radicals cause lipid peroxidation and necrosis of hepatocytes [4].

Parkinsonia Aculeata contains tannins, saponins, glycosides, alkaloids, steroids and volatile oils [4]. Hepatotoxic markers studied in rats include SGOT, SGPT, hepatic triglycerides together with histopathological examination [6].

Today research is also directed towards exploring potential of herbal medicines as new remedial measures to treat different ailments [1]. Steroids, vaccines and antiviral drugs which have been employed as therapies for liver diseases have potential adverse effects, especially when administered for long terms. Therefore, herbal products and traditional medicines with improved effectiveness and safety profiles are needed as a substitute for chemical therapeutics [8]. Traditional medicine is used in the maintenance of health, as well as in the prevention, diagnosis, improvement or treatment of physical and mental illnesses. In many developed countries, 70 to 80% of the population is using some form of alternative or supportive medicine. Especially in some Asian and African countries like India, more than 80% of people depend on plant based traditional medicine for primary health care [WHO, 2002]

Herbs are useful for protecting various organs owing to their potential antioxidant constituents such as quercetin β -carotene, tocopherol and Vit-C. *P. aculeata* is a small, spiny tree, belonging to the family Leguminosae and is native to tropical America, extending from Mexico to South America [5]. It grows 2 to 8 m (6.6 to 26 ft) high, with a maximum height of 10 metres (33 ft). Leaves bipinnate, ending in a stout spine. It is often branching near the ground with a very open crown of spreading branches and very thin drooping foliage. It remains green throughout the year and appears leafless after leaflets fall [5]. Common names include Palo verde, Mexican palo verde, Parkinsonia, Jerusalem thorn In India it is found in all dry regions, particularly western parts. Plantations of this species are also being raised in the arid and semi-arid tracts of western Uttar Pradesh, Rajasthan and Gujarat.



MATERIALS AND METHODS

Ethics committee approval

Experimental protocols were reviewed and approved by IEC of Y.B.Chavan College of Pharmacy Aurangabad.

Chemicals

CCl₄ of analytical grade was used.

Collection of plant material

The leaves of *P. aculeata* were collected from outskirts of Aurangabad city and authenticated by Department of Botany, Maulana Azad College of Arts, Science and Commerce Aurangabad.

Preparation of extract

The leaves were shade-dried at room temperature and different extracts of *P. aculeata* were obtained using Soxhlet apparatus.

Drug Formulation

The different extracts of plant leaves were suspended in distilled water using Tween 40.

Experimental Animals

The experiments were carried out on Sprague-Dawley (150–200 gms) rats of either sex. Animals were maintained in standard laboratory conditions of temperature and humidity. The rats were fed on standard food pellets and water *ad libitum*.

Phytochemical analysis

The extracts of leaves were subjected to preliminary phytochemical screening to identify the presence of various phytoconstituents present. It showed the presence of Alkaloids, glycosides, saponins, flavanoids etc.

Experimental Induction of Hepatotoxicity

Liver toxicity was induced in rats by administering carbon tetrachloride (CCl₄)

intraperitoneally (i.p) , with Arachis oil (1: 1 v/v) at the dose of 1 ml/kg body weight of (CCl₄) for two days .

Experimental Design

After acclimatization the rats were divided in to seven groups of six rats each of either sex.The method was slightly modified and pretreatment with leaf extracts was done for three days prior to induction of Hepatotoxicity with CCl₄.

Animals of **Group 1** were treated as normal control group.

Animals of **Group 2** (CCl₄ control group) were treated with CCl₄ administered (1ml/kg,i.p) diluted in Arachis oil (1:1) for two days.

Animals of **Group 3** were treated initially with Standard drug Liv 52 for 3 days in a dose of 1ml/kg orally ,then with CCl₄ : Arachis oil(1:1) (1ml/kg i.p CCl₄) and Liv 52 simultaneously for two days and only Liv 52 for remaining five days.

Animals of **Group 4** were treated initially with Pet Ether extract for 3 days in a dose of 500mg/kg orally, then with CCl₄ : Arachis oil (1:1) (1ml/kg i.p CCl₄) and extract simultaneously for two days and only Pet Ether extract for remaining five days.

Animals of **Group 5** were treated initially with Chloroform extract for 3 days in a dose of 500mg/kg orally, then with CCl₄ : Arachis oil (1:1) (1ml/kg i.p CCl₄) and extract simultaneously for two days and only chloroform extract for remaining five days.

Animals of **Group 6** were treated initially with Methanol extract for 3 days in a dose of 500mg/kg orally, then with CCl₄ : Arachis oil (1:1) (1ml/kg i.p CCl₄) and extract simultaneously for two days and only Methanol extract for remaining five days.

Animals of **Group 7** were treated initially with Aqueous extract for 3 days in a dose of 500mg/kg orally, then with CCl₄ : Arachis oil (1:1) (1ml/kg i.p CCl₄) and extract simultaneously for two days and only Aqueous extract for remaining five days.

Hepatoprotective activity

Overnight fasted rats were used. Animal was anaesthetized by using mild ether and 1% of sod.citrate was injected (i.p.) to prevent blood clotting [1]. Rat was sacrificed by cervical dislocation and blood sample was collected by cardiac puncture (cardiac pouch) for evaluating the biochemical parameters & a portion of the median lobe of the liver was dissected and fixed in 10% neutral buffered formalin solution for 24 h. Liver tissue slices were collected for histopathological studies.Biochemical parameters like serum glutamate oxaloacetate transaminase (SGOT) & serum glutamate pyruvate transaminase (SGPT) and triglycerides were assayed according to the standard method

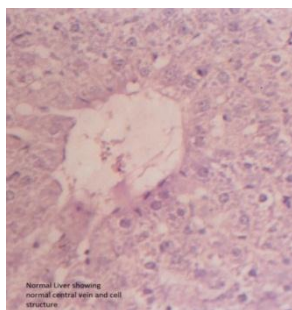
Thiopental Induced sleeping time in rats

Pentobarbital induced sleeping time was evaluated according to the method previously described by [2]. Thiopental sodium was used instead of Pentobarbitone for its short duration

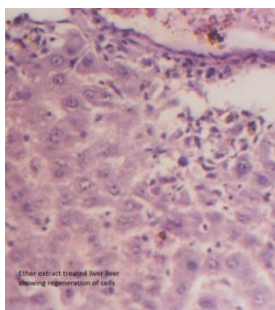
of action. The dose of Thiopental sodium was (25 mg/kg i.p) [3] .The standard drug taken was Diazepam (1mg/kg i.p).The various extract treated animals were used and the animals were observed for time taken for sleep induction and duration of sleep.

Acute Toxicity Studies

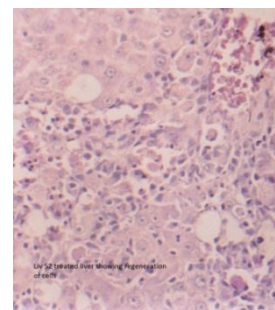
From the acute toxicity study it was observed that oral administration of all four extracts at 2000 mg/kg did not cause mortality in the animals. The extract was found safe in animals dosed at 2000 mg/kg indicating the LD₅₀ above 2000 mg/kg.



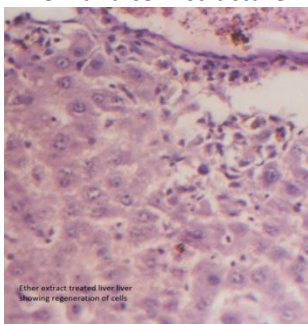
1. Normal liver showing normal central vein and cell structure



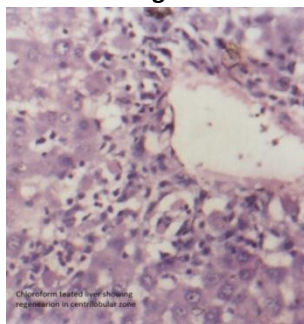
2. Control liver showing central vein hemorrhage and necrosis



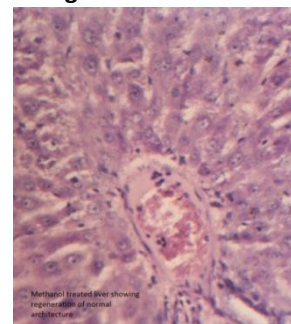
3. Liv s2 treated showing regeneration of cells



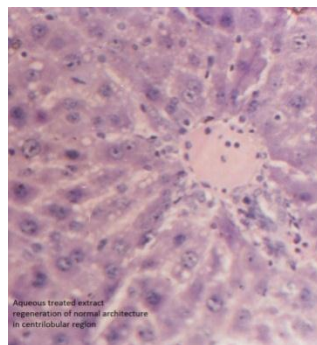
4. Ether extract treated liver showing significant regeneration of cells



5. Chloroform ext treated liver showing slight regeneration in centrilobular zone



6. Methanol treated liver showing significant regeneration normal architecture



7. Aqueous extract treated liver ,no significant regeneration of normal architecture in centrilobular region

Table No.1 Estimation of Bio Chemical Parameter in CCL4 induced Hepatotoxicity in rats

Groups	Dose mg/kg	SGOT	SGPT	Triglyceride
Group I(Normal)		139+ 4	59.1 +1.8	54.3+1.9
Group 2(Control)	CCL4 (1ml/kg)	315.6+7	197+5.2	113+4.5
Group 3 (Standard)	Liv 52(1ml/kg)	172.3± 1.9	64+1.5	109.3+1.5
Group 4 (Ether)	500 mg/kg	144 + 2.7	92.3+2.1	52.6+1.6
Group 5 (Chloroform)	500 mg/kg	202.6 ±3.6	64.3+2.3	95.3+1.6
Group 6 (Methanol)	500 mg/kg	174 + 2.2	99.6+15.3	50.6+1.1
Group 7 (Aqueous)	500 mg/kg	214 +8.7	96.6+2.3	54+0.8

Values are mean± S.E.M, n=6, p value ≤ .001 compared to CCL4 control

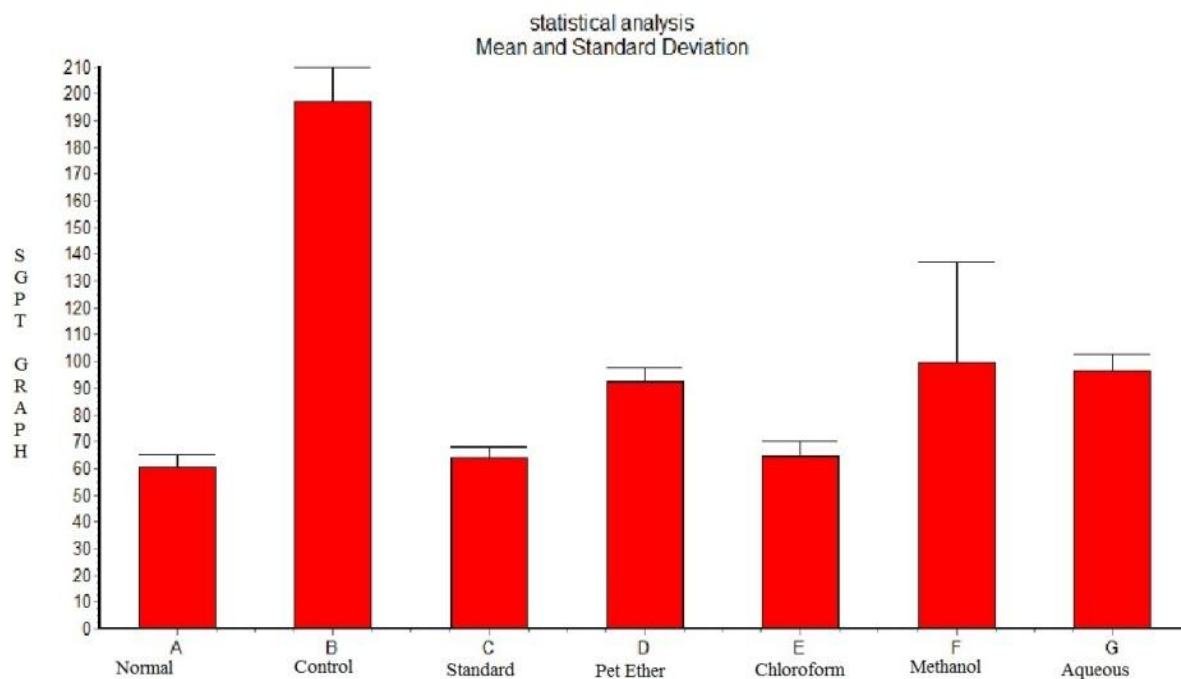
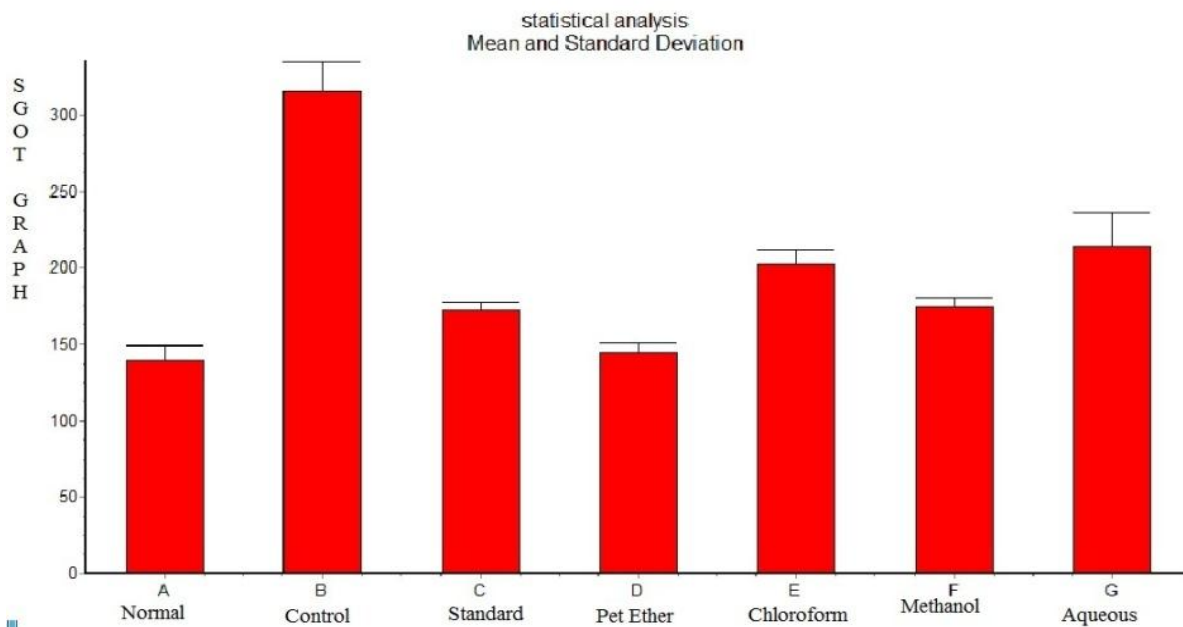
Table No.2 Potentiation of Thiopental Induced Sleep duration

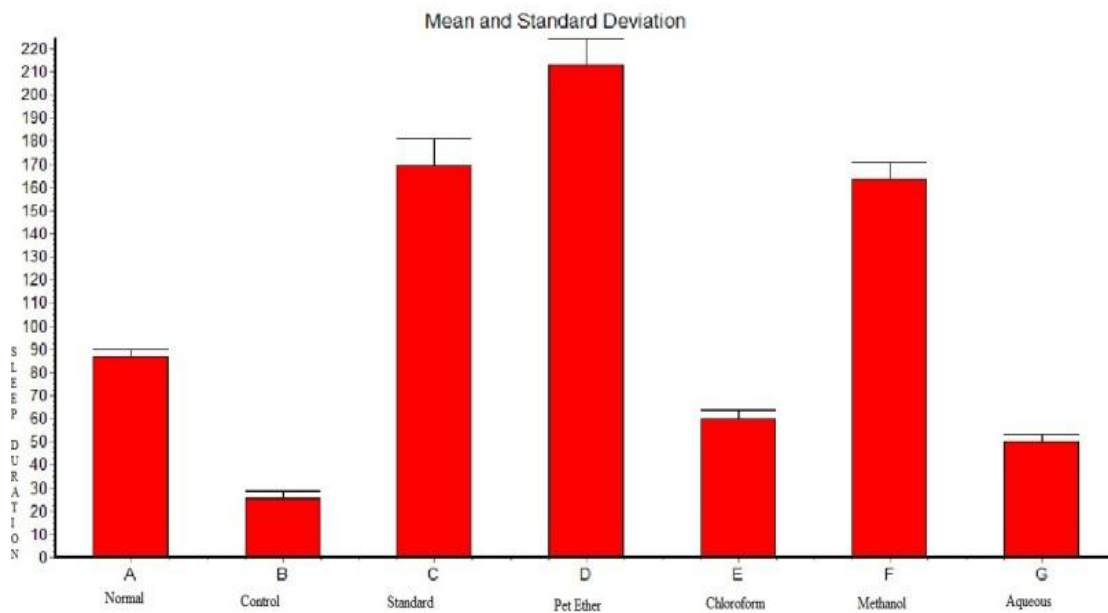
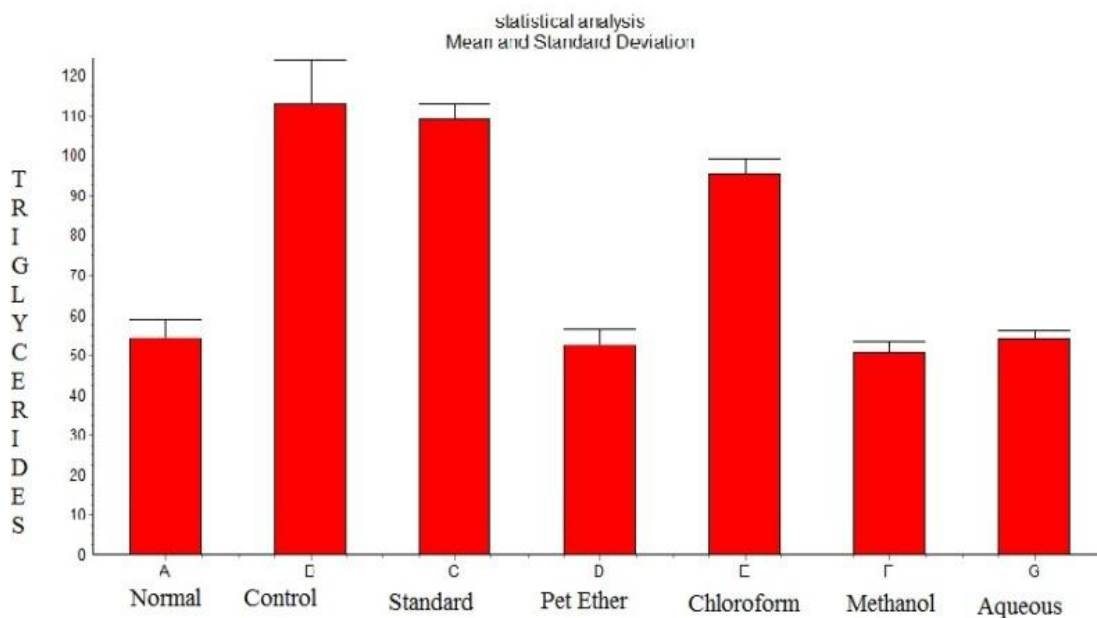
Groups	Onset of Sleep (mins)	Duration of Sleep (mins)
Group I(Normal)	21±1.04	86± 1.2
Group 2(Control)	12.3± 0.72	25± 1.1
Group 3 (Standard)	6± 0.28	168± 4.8
Group 4 (Ether)	15.6± 1.7	213± 4.4
Group 5 (Chloroform)	35± 1.4	60± 1.4
Group 6 (Methanol)	29± 1.04	163± 3
Group 7 (Aqueous)	42± 4.8	50± 1.4

Values are mean± S.E.M, n=6, p value ≤ .001 compared to CCL4 control

Statistical Analysis

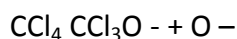
The results of the biochemical estimations are reported as mean ± SEM of six animals in each group. The data were subjected to one-way analysis of variance (ANOVA). This was followed by Post Hock Tukey test, to determine the statistical significance of the difference in enzyme activity and other parameters. The level of significance was P < 0.001.





DISCUSSION

Popularity of herbal remedies is increasing globally and atleast on quarter of patients with liver diseases use ethonobotanicals [9].The present study reports the potential hepatoprotective activity of *P. aculeata* against hepatic injury produced by CCl_4 in rats. The changes associated with CCl_4 induced liver damage are similar to that of acute viral hepatitis[13].The mechanism of CCl_4 induced liver injury is due to the lipid peroxidation caused by the free radical derivatives of CCl_4 [4]. CCl_4 is metabolized in endoplasmic reticulum and mitochondria with the formation of CCl_3O by cytochrome P-450. The nascent oxygen O- causes rise in intracellular reactive Fe^{2+} ions, aldehyde and depletion of GSH, and calcium sequestration via lipoperoxidation.



An obvious sign of hepatic injury is the leaking of cellular enzymes into the plasma The estimation of enzymes in the serum is a useful quatitative marker of the extent and type of hepatocellular damage[13].In the present study, a significant increase in the activities of SGOT, SGPT and Triglycerides due to exposure to CCl_4 was observed, indicating considerable hepatocellular injury[4].

The plant kingdom appears to be an important resource of phytochemicals and hepatoprotective properties(4). Leaves of *Parkinsonia aculeata* Linn. reported to contain C-glycosides (epi-orientin, Parkinsonin-A, Parkinsonin-B, Parkintin) and flavone C-glycoside (Luteolin), orientin, iso-orientin, vitexin, iso-vitexin, lucenin-II, vicenin-II, diosmetin 6-C- β -glucoside, apigenin, luteolin, kaempferol and chrysoeriol [1]. It has been reported that *P. aculeata* has potent antioxidant activity.

From our present results CCl_4 caused an abnormal reduction in the percentage of viability of hepatocytes, while abnormal increase in the SGOT, SGPT and Triglycerides levels. Pretreatment with Pet Ether and Methanol extract significantly reversed all these abnormal changes and thus offered protection against CCl_4 induced toxicity to rat hepatocytes. Pet Ether Extract (at 500 mg/kg) normalized the viability of hepatocytes as well as cytosolic enzymes to near normal indicating that Pet Ether extract and Methanol (at 500 mg/kg) concentration is effective in reversing CCl_4 induced toxicity to rat hepatocytes.Further the stimulation of hepatic regeneration was known to make the liver more resistant to damage by toxins[13]. The present study proves the potential hepatoprotective activity of *P. aculeata* and gives insight into its mechanism of action. Possible mechanism that may be responsible for the protection of CCl_4 induced liver damage by Pet Ether and Methanol extract include potent antioxidants and to the presence of tannins, flavanoids, saponins, glycocides, alkaloids, steroids and volatile oils in the extracts(4).Reduction in the levels of SGOT and SGPT towards the normal value is an indication of stabilization of plasma membrane as well as hepatic tissue damage caused by CCl_4 [11]. So the extracts could act as a free radical scavenging agents. Therefore, Pet Ether and Methanol extract is a promising hepatoprotective agent. The hepatoprotective action combined with antioxidant activity has a synergistic effect to prevent the process of initiation and progress of hepatocellular damage. The pre treatment of animals with plant extracts resulted in

prolongation of Thiopental sleeping time ($p < .001$) therefore it is conceivable that the plant extract might contain MDME (microsomal drug metabolizing enzymes) inhibitory constituents that cause hepatoprotection [12].

Histopathology Findings

CCl_4 is a hepatotoxicant known to produce a characteristic centrilobular pattern of degeneration and necrosis [7](slide 2). The histopathology studies of liver showed fatty changes, swelling and necrosis with loss of hepatocytes in CCl_4 control rats in comparison with normal control (slide 1). The Pet Ether and Methanol treated groups showed regeneration of hepatocytes, normalization of fatty changes and necrosis of the liver (slide 4 and slide 6 respectively), the Liv 52 treated group (slide 3) also showed normalization of fatty accumulation and necrosis. The maximum protection against hepatic damage was achieved with leaf extract of Pet Ether and Methanol extract.

CONCLUSION

Chronic hepatic diseases stand as one of the foremost health troubles worldwide, with liver cirrhosis and drug induced liver damage accounting ninth leading cause of death in western and developing countries. Therefore, treating liver diseases with plant derived compounds which are accessible and do not require laborious pharmaceutical synthesis seems highly attractive [9].

The present study indicates that *P. aculeata* has marked antioxidant activity due to presence of flavonoids which may act in a similar fashion as reductones by donating the electrons and reacting with free radicals to convert them to more stable product and terminate free radical chain reaction [10]. The present studies indicated that the extracts of leaves of *P. aculeata* possess potent hepatoprotective activity comparable to that of standard Liv 52 which is known hepatoprotective agent as evidenced by the serum biochemical parameter and histopathological studies and sleeping time induced by Thiopental. The plant would be useful for treatment of various diseases mediated by free radicals [10].

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