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Effect of *Azadirachta indica* leaf extract on carbontetrachloride-induced hepatic damage in albino rats

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The shade dried leaves of *Azadirachta indica* were extracted successively by petroleum ether (60-80°) and ethanol. The ethanol extract after removal of solvent was studied for carbontetrachloride induced hepatic changes using 0.5 g and 1 g/kg oral dose in albino rats. The changes were assessed by serum enzyme profile that include glutamic oxaloacetate transaminase (GOT), glutamic pyruvic transaminase (GPT), alkaline phosphatase (AP), bilirubin (B) and hepatic triglycerides (HTG) levels, histological changes in liver and pentobarbitone sleeping time as a functional parameter. There was significant reversal of biochemical, histological and functional changes induced by carbontetrachloride in rats by ethanol extract treatment.

AZADIRACHTA indica A. Juss (Neem) of family Meliaceae is native to Indian sub-continent. Now-a-days it is grown in many Asian countries and tropical region of the Western hemisphere. Since ancient times, seeds and leaves of this tree have been used traditionally to cure various human ailments and as a household pesticide[1]. Different parts of this plant have been reported to possess various medicinal properties[2,3]. Leaves of this plant are traditionally known or reported for various biological activities like antiinflammatory[4], anxiolytic[5], antiandrogenic[6], antistress[7], humoral and cell mediated immunostimulant[8], anithyperglycmic[9], liver stimulant[10,11] antiviral[12] and antimalarial[13]. Traditionally decoction of leaves of this plant is used for jaundice and liver complaints in Sagar district of Madhya Pradesh[14]. In view of this, it was proposed to screen leaf extract on carbontetrachloride(CCl_4) induced hepatotoxicity in rats. CCl_4 is well known liver toxin and it causes structural, functional and compositional changes in liver[15].

MATERIALS AND METHODS

Plant material : The leaves of *A. indica* were collected from Pune region in bulk quantity and shade dried. These dried leaves were coarsely powdered and defatted using petroleum ether (60-80°), subsequently extracted exhaustively in a soxhlet apparatus with ethanol. This extract (ET) was dried at low temperature and pressure (yield 6.99%). It showed presence of flavonoids, saponins, tannins in preliminary phytochemical tests.

Albino rats (HA strain) of either sex weighing between 80-120 g were obtained from the animal colony of Agharkar Research Institute (ARI) for experimental purpose. They were housed in an air-conditioned area at 25±2° with 10:14 hours light and dark cycle. The animals were maintained on Amrut brand pellet feed and water *ad libitum*. They were kept fasting 24 hours prior to experimentation.

ET was administered orally upto 2 g/kg to individual rat in a group. There was no mortality due to the above treatment. Hence, for further studies 1 g/kg per oral maximum dose was employed.

Initial pilot studies were performed using various doses of CCl_4 as well as extract using three rats per dose at various time intervals. The criteria for the selection of dose and the time interval for hepatotoxicity and reversal of

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Table No. 1: Effect of ET on pentobarbitone sleeping time in albino rats

Group No.	Treatment	Mean sleeping time in minutes ± SE.
1	Control	37.00 ± 3.44
2	CCl_4	73.25 ± 4.19*
3	ET-0.5 g/kg	42.00 ± 3.46**
4	ET-1 g/kg	42.25 ± 3.74**
5	CCl_4+ET-0.5 g/kg	62.75 ± 4.47 @@
6	CCl_4+ET-1 g/kg	56.00 ± 2.02 @

1. * Significant, ** Non-significant as compared to control.

2. @ Significant, @@ Non-significant as compared to CCl_4 treatment group only.

hepatotoxicity by CCl_4 with extract respectively were functional, biochemical and histopathological changes induced by CCl_4 in rats.

Effect on pentobarbitone-sleeping time in albino rats : Pentobarbitone was administered in a dose of 35 mg/kg by intraperitoneal route to albino rats for this study. Time interval between loss and regain of righting reflex was measured as sleeping time. A group of 48 rats was divided randomly in 6 groups of 8 rats each. The effect of oral dose of ET 0.5 g and 1 g/Kg was studied 24 hours after subcutaneous treatment of 1.50 ml/kg CCl_4 in olive oil (1:1) to assess effect on pentobarbitone-induced hypnosis using corresponding control as shown in Table No. 1.

Effect on CCl_4 induced hepatotoxicity : In this study CCl_4 in a dose of 2 ml/kg in olive oil (1:1) was administered subcutaneously as hepatotoxic agent. A group of 36 rats was divided randomly into 6 groups of 6 each. The treatment of ET was given in a dose of 0.5 g and 1 g/kg by oral route for 8 days. Subsequently, after 8th day treatment, blood from each rat was collected through cardiac puncture under ether anaesthesia. The serum was separated for estimating the following; glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT)[16], alkaline phosphatase (AP)[17], bilirubin (B)[18] and hepatic triglyceride (HTG)[19] was estimated from 1 g liver homogenate in 10 ml normal saline.

The liver from each animal was removed after dissection. The liver lobes were fixed for 48 hours in 10% formalin and were embedded in paraffin. Subsequently, 5 μ sections were cut on a microtome and stained with haematoxylin and eosin. These sections were observed under light microscope for histological changes and compared to normal liver histology.

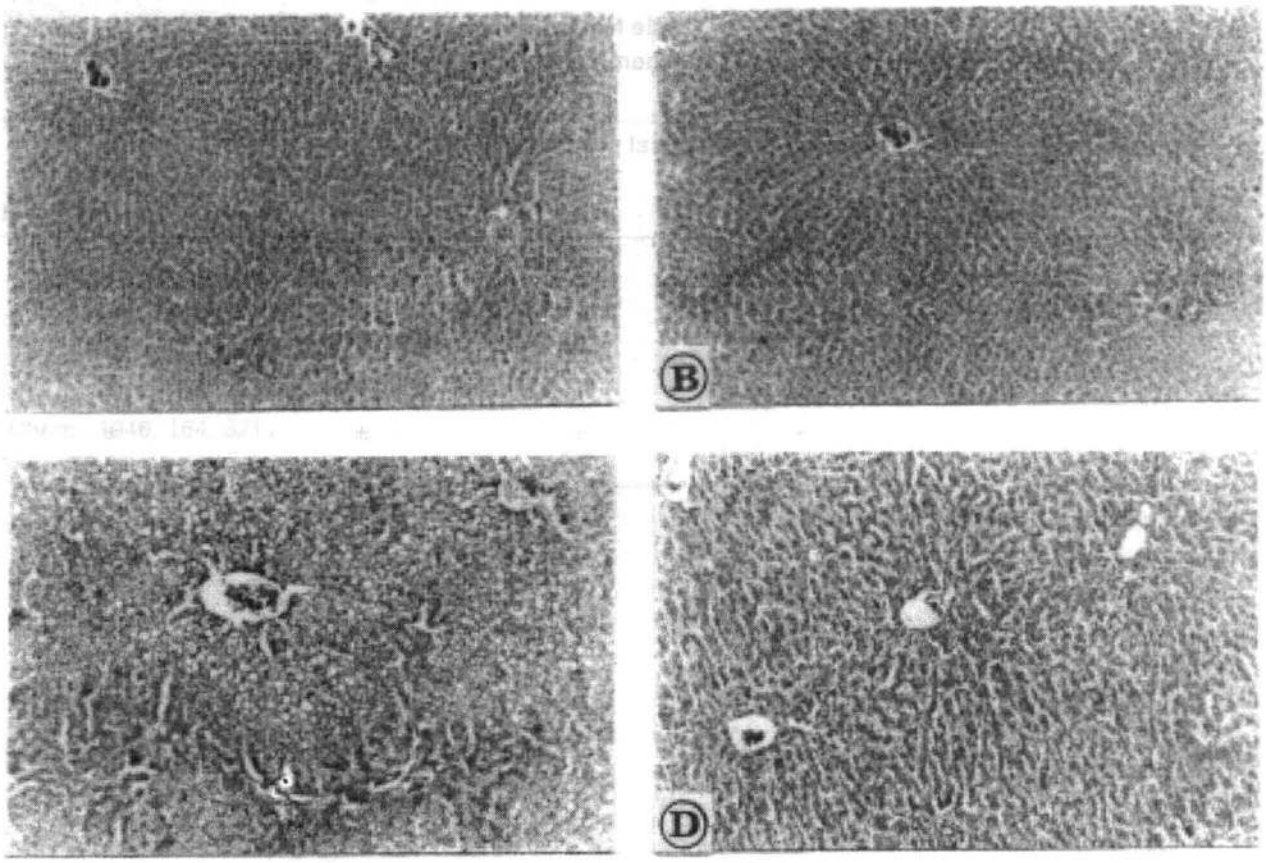
Statistical analysis : All results of pentobarbitone sleeping time and biochemical estimations were reported as mean ± SE. These studies were further analysed by using students t test to calculate significance of the results. The p values greater than 0.05 were considered as non-significant.

RESULTS

Effect on pentobarbitone-sleeping time : The effect of 0.5 g and 1 g per kg of ET by oral route was studied on pentobarbitone sleeping time, with CCl_4 and without CCl_4 pretreatment. The results are shown in table No. 1. There is no significant effect on sleeping time due to ET pretreatment prior to pentobarbitone. Further there is dose-dependent reduction in sleeping time due to ET treatment in CCl_4 pretreated rats. However, this value is significant at higher dose only.

Effect on CCl_4 induced hepatotoxicity in rats - The effect of ET treatment on normal and CCl_4 pre-treated rats for 8 days on various biochemical parameters are shown in table No. 2. There is no alteration in the biochemical profile studied in control and ET treated rats. As shown in table No. 2 there is significant alteration in all biochemical parameters due to CCl_4 treatment only. There is reduction in all biochemical parameters in CCl_4 pretreated groups due to ET treatment. However, these values are significant at 1 g/kg dose of ET only.

The histopathological profile of liver of CCl_4 treated rats is shown in plate which showed intense characteristic centrilobular necrosis and vacuolization with fatty degeneration. There was scanty mononuclear infiltration in the area of fatty degeneration. However, the extract treated animals which are pretreated with CCl_4, showed focal microvesicular fatty degeneration, with mononuclear cell infiltration and focal spotty necrosis. Thus, extent of damage caused by CCl_4 is reduced due to ET treatment. The hepatic cells are not altered by ET treatment alone.



Effect of ET treatment on CCl_4 induced hepatic histopathological changes in albino rats

(A) Control, (B) ET 1 g/kg treatment, (C) CCl_4 induced hepatic changes, (D) Reversal of CCl_4 induced hepatic changes by ET-1 g/kg treatment

DISCUSSION

Liver is a versatile organ in the body concerned with regulation of internal chemical environment. Therefore, damage to the liver inflicted by hepatotoxic agents is of grave consequence. There is an ever increasing need for an agent which could protect liver from damage, especially of one which facilitates regeneration by proliferation of parenchymal cells after damage and arrest growth of fibrous tissue[20]. As such liver is highly affected primarily by toxic agents such as CCl_4, paracetamol, D-galactosamine, alcohol and thioacetamide through different mechanisms[21].

In the present study, CCl_4 is used as a hepatotoxic agent. It is well established that hepatotoxicity by CCl_4 is due to enzymatic activation to release CCl_3 radical in free state, which in turn disrupts the structure and function of lipid and protein macromolecules in the membrane of the cell organelles[22,23]. After treatment with CCl_4 there is significant rise in serum GOT, GPT, bilirubin and alkaline phosphatase levels as compared to control animals. This enzyme profile is altered in dose dependant manner due to ET treatment, however, it is significantly lowered only at 1 g/kg dose. This finding is further supported by histopathological observations. These observations are in corroboration with the earlier work carried out by Chattopadhyay *et al*[11] using paracetamol as hepatotoxicant. It is known that CCl_4 enhances lipid peroxidation leading to accumulation of triglycerides in hepatic parenchyma cells[24,25] a step further leads to necrosis[26]. In the present study, hepatic triglyceride levels are significantly reduced in CCl_4 pre-treated animals due to ET treatment. Effect on pentobarbitone sleeping time is taken as a functional index for the microsomal enzyme activity. The ET has shown reduction in sleeping time and shown protection against CCl_4 induced potentiation of sleeping time. Thus, all these

Table No. 2

Effect of ET treatment on various biochemical parameters in CCl_4 pre-treated rats

Group No.	Treatment	Various biochemical parameters ± SE				
		GOT (U)	GPT (U)	B (mg/l)	AP (U)	HTG (mg/g wet wt)
1.	Control	74.99 ± 4.93	51.63 ± 2.81	2.65 ± 0.11	78.48 ± 2.42	16.62 ± 0.84
2.	CCl_4	99.11* ± 4.95	69.29* ± 3.11	12.87* ± 0.51	106.59* ± 2.71	38.70* ± 2.97
3.	ET-0.5 g/kg	69.05** ± 2.07	50.28** ± 2.55	2.76** ± 0.16	78.89** ± 1.85	14.91** ± 0.83
4.	ET-1 g/kg	75.34** ± 2.18	46.40** ± 1.97	2.91** ± 0.20	80.22** ± 1.51	13.59** ± 0.45
5.	CCl_4 + ET 0.5 g/kg	91.57@@ ± 2.60	63.40@@ ± 3.10	11.84@@ ± 0.31	102.75@@ ± 3.60	35.85@@ ± 2.19
6.	CCl_4+ET 1 g/kg	83.34@ ± 4.70	60.28@ ± 2.56	8.76@ ± 0.45	88.34@ ± 2.14	28.12@ ± 1.72

* Significant, ** Non-significant as compared to control.

@ Significant, @@ Non-significant as compared to CCl_4 treatment only.

findings together conclude that ET has biochemical, histopathological and functional protection against CCl_4-induced hepatic damage.

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