USE OF THE LEAVES SLURRY OF *NYCTANTHES ARBOR TRISTIS LINN*. FOR HEPATOSUPPERATION INDUCED BY CARBON TETRA CHLORIDE IN WISTAR ALBINO RATS.

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Summary

The present study deals with the amelioration by *Nyctanthes arbor tristis linn*. leaves slurry against hepatosuppression induced by carbon tetrachloride (CCl_4), which was evaluated in terms of serum marker enzymes like viz. GOT, GPT, Alkaline phosphate, glucose, cholesterol, and total protein concentration in blood. These biochemical parameters were significantly ($P< 0.001$) altered by the single dose of CCl_4 (0.7 ml/kg) pretreatment with *Nyctanthes arbor tristis linn*. Prior to the administration of CCl_4, at the doses of 0.5g/kg.body weight /day, P.O. for 3 days, significantly restored all the serum and liver parameters near to the normal levels, respectively. However, silymarin was used as a reference standard, prior to the administration of CCl_4 to rats. These findings indicate the hepatoprotective potential of *Nyctanthes arbor tristis linn*. against hepatosuppression possibly involve mechanism related to its ability to block the *P*-450 mediated CCl_4 bioactivation through selective inhibitors of ROS (reactive oxygen species) like antioxidants brought about significant inhibition of TBARS suggesting possible involvement of O_2 –, HO_2, HO_2 –, H_2O_2 and OH. . Thus *Nyctanthes arbor tristis linn*. showing protection in liver may prove promising as a rich source of antioxidants because its use is cost effective, especially for peoples in adverse and hazardous circumstances, who are living in poverty.

Key words : *Nyctanthes arbor tristis linn*, CCl_4, marker enzymes, hepatoprotection.

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Introduction

Nyctanthes arbor tristis linn widely found throughout India is known in Indian traditional medicine to posses immunotoxic [1], antiallergic [2], antihistaminic and purgative [3], antibacterial and cytotoxicity [4], antipyretic and ulerogenic [5 and anti - Inflammatory [6] activity. Despite extensive research in medical field, no drug in the modern system of medicine can be claimed to cure liver disorders, which in many cases becomes fatal. Although plant extracts of *Picrorrhiza kurroa, Andrographics paniculata, Eclipta Alba*, [7] and *B.Valgaris* [8] have been reported to possess clinically useful hepatoprotective activity, many plants remain unexplored.

At present, one of the plant-derived medicines approved for use in liver cirrhosis and alcoholic liver diseases is silymarin. There are number of studies which conclude the efficacy of silymarin in these conditions [9]. Silymarin is a mixture of flavonolignans from the fruits of *Silybum marianum* that has been known since ancient time and recommended in traditional European and Asian medicine mainly for the treatment of liver disorder [10]. Therefore in the present study silymarin was used as positive control to compare the efficacy of *Nyctanthes arbor tristis linn* against CCl_4-induced hepatotoxicity.

Materials and Methods

Plant

Nyctanthes arbor tristis linn leaves were collected from the fields in and around Ahmednagar (District), Maharastra, India and authenticated by the Botanical Survey of India, Pune. A voucher specimen is deposited in the Department of Organic Chemistry in Sangamner College, Sangamner.

Preparation of leaves powder slurry

Leaves dried at 40^0C and crushed to make fine powder. A portion of the leaves powder was dissolved in distilled water, filtered and dried to determine the amount of the water-soluble fraction in the residue. Prior to the experiment 0.5gm leaves powder was dissolved in a saline/Cremophor (0.025%v/v) solution and diluted to desired concentration to give a water soluble fraction (AFSC).

Animals

Wistar rats of either sex, weighing 150–250 g, were used. Animals were housed under controlled conditions of temperature (25 ± 2^0C) and photoperiod 12-h light/dark and fed with standard rodent pellet diet with tap water.

Induction of hepatic injury

Hepatic injury was induced in rats by subcutaneous administration of a single dose of 0.7ml/kg $CC1_4$ mixed with 0.5ml liq. Paraffin on the 7th day, 2 h after the last treatment [11].

Experimental protocol

Animals were grouped as follows:
Normal Control group: Treated with vehicle (1.0ml,liq.paraffin i.p.) on first day. Followed by 2ml D/W daily for 3 days.
CCl_4 control group: Treated with 0.7ml/kg CCl_4 in 1.0ml. Liq.paraffin i.p. Followed by 2.0ml D/M water oral dose daily for 3 days.
CCl_4 Recovery group: Treated with 0.7ml/kg CCl_4 in 1.0ml. Liq.paraffin i.p on first day, Followed by 2.0ml D/M water oral dose daily for 7 days.
Silymarin control group: Treated with 0.7ml/kg CCl_4 in 1.0ml. Liq.paraffin i.p. and 0.07g/kg silymarin daily for 3 days.

Leaves powder control: Treated with 0.7ml/kg CCl_4 in 1.0ml. Liq.paraffin i.p.and 0.5g/kg leaves powder slurry dose daily for 3 days.
On day 4, 48 h after $CC1_4$ administration, blood sample of each animal was taken from abdominal aorta under pentobarbitone anesthesia (350 mg/kg i.p.) and serum cholesterol [12], GOT, GPT, Bilirubin [11], serum glucose, total protein [12] and alkaline phosphates [13] were evaluated. Also histological studies are done under light microscope and electeron microscope [18-19].

Statistical analysis

All values are expressed as means ±S.D. The results were calculated and subjected to analysis of variance (ANOVA) considered significant [14].

Results

Food consumption and weight gain

We observed that there was significant decrease in body weight of CCl_4 treated group as compared to normal control group. Treatment of rats with silymarin and leaves powder slurry showed significant increase in body weight as compared to CCl_4 treated group (Table 1).

Table 1. Effect o*f Nyctanthes arbor tristis linn* leaves powder on body weight

Groups	Body weights of rats (g)[#]						
	1st Day	2nd Day	3rd Day	4th Day	5th Day	6th day	7th Day
Normal control	160.9± 3.2	161.33± 3.3	161.5± 3.5	162± 3.6	162.9± 3.2	163.9± 4.2	164.5± 2.6
CCl_4 control	155.1± 4.2**	146.2± 4.6*	140.2± 5.0**	135.0± 5.0*	130± 5.1**	127.5± 4.5*	122.9± 5.0**
CCl_4 recovery	154.1± 5.0*	156.5± 4.5**	158.5± 4.8***	158.9± 5.0*	159.2± 5.2**	160.1± 3.2**	160.2± 4.3**
Silymarin control	158.1± 3.2***	160.2± 9.0***	161.1± 6.2**	161.5± 7.5***	162.0± 8.0***	162.5± 3.2***	163.0± 3.6***
Leaves powder control	160.1± 4.0**	160.5± 3.2**	161.8± 4.0**	161.8± 4.5**	162.2± 3.2**	162.9± 2.6**	163.2± 5.9***

N=6 [#]Values are expressed as mean of ± S.D.***P <0.001, **P <0.01, *P <0.05 in comparison to Normal control group.

Serum marker enzymes
All the marker enzymes, *viz.*, AST, ALT, ALP and GGT registered enhanced activity in CCl_4-treated rats as compared to control group (Table 2). In MEC co-administered group, the levels of these enzymes were found retrieving towards normalcy.

Table 2. Effect of *Nyctanthes arbor tristis linn* leaves powder on CCl_4-induced liver damage in rats

Groups	BILI	GOT	GPT	Alk-PO_4	Serum Glucose (mg/dl)	Serum cholesterol (mg/dl)	Total protein
Normal control	0 525 ± 0.03	157.89± 19.31	92.24± 4.46	147.75± 9.07	146.44± 11.55	72.14± 5.16	5.65± 0.6
CCl_4 control	0.49± 0.03	247.06± 14.26**	184.4± 12.11***	171.33± 11.39	180.47± 6.92	73.61± 2.59	3.29± 1.38**
CCl_4 Recovery	0.53± 0.02	217.98± 27.35*	155.83± 12.61***	171.33± 20.69	222.73± 14.8*	71.79± 6.2	2.26± 0.20***
Silymarin control	0.53± 0.03	248.75± 20.18**	179.83± 15.91***	141.33± 7.37***	190.9± 18.15	61.11± 7.30	6.43± 0.70
Leaves powder control	0.47± 0.05	186.83± 15.62*	165.33± 13.11*	128.83± 6.58***	249.40± 21.6**	78.63± 3.02	4.11± 0.35*

N=6,Values are expressed as mean of ± S.D.***P <0.001, **P <0.01, *P <0.05 in comparison to Normal control group.

Other biochemical parameters:

The total protein concentration of the serum and liver was lesser in Group II animals, when compared with normal control. (Tables 3) and it attained an almost normal value in group III rats. The level of total lipids, triglycerides and cholesterol in serum as well as liver recorded significant increment in CCl_4- administered rats as compared to those of group I. All these biochemical changes showed signs of returning towards the normalcy in-group III animals. There was a significant decline in the concentration of phospholipids in liver tissues of CCl_4-treated rats as compared to normal control. In group III animals phospholipid concentration attained normalcy.

Table 3. Effect of *Nyctanthes arbor tristis linn* leaves powder on liver weight and volume

Groups	Liver Weight# (gm)	Liver Volume# (ml)
Normal control	6.32± 0.05	7.95± 0.05
CCl_4 control	7.752± 0.04 **	11.01± 0.07***
CCl_4 Recovery	7.269± 0.06**	9.05± 0.07***
Silymarin control	6.63± 0.09***	8.45± 0.05**
Leaves powder control	6.07± 0.10**	7.75± 0.12**

N=6 #Values are expressed as mean of ± S.D.***P <0.001, **P <0.01, *P <0.05 in comparison to Normal control group.

Histological studies

After sacrifice of rats the pieces of liver were fixed in aqueous Bouin's fixative for 24 hours. The tissues after fixation were washed in water to remove excess of fixative. Washed tissues were then dehydrated through graded series of ethyl alcohol, cleared in xylene and embedded in paraffin. Sections were cut at 5 to 7 pm and mounted on clean glass slides. The sections were routinely stained with Hematoxylin and Eosin technique. Slides were observed under light microscope and electeron microscope and photographs were taken and attached here Fig-1, Fig-2, Fig-3, Fig-4, Fig-5are observed under light microscope and Fig-6, Fig-7, Fig-8, Fig-9, Fig-10 are observed under electeron microscope.

Fig.- 1 (Normal Control group)

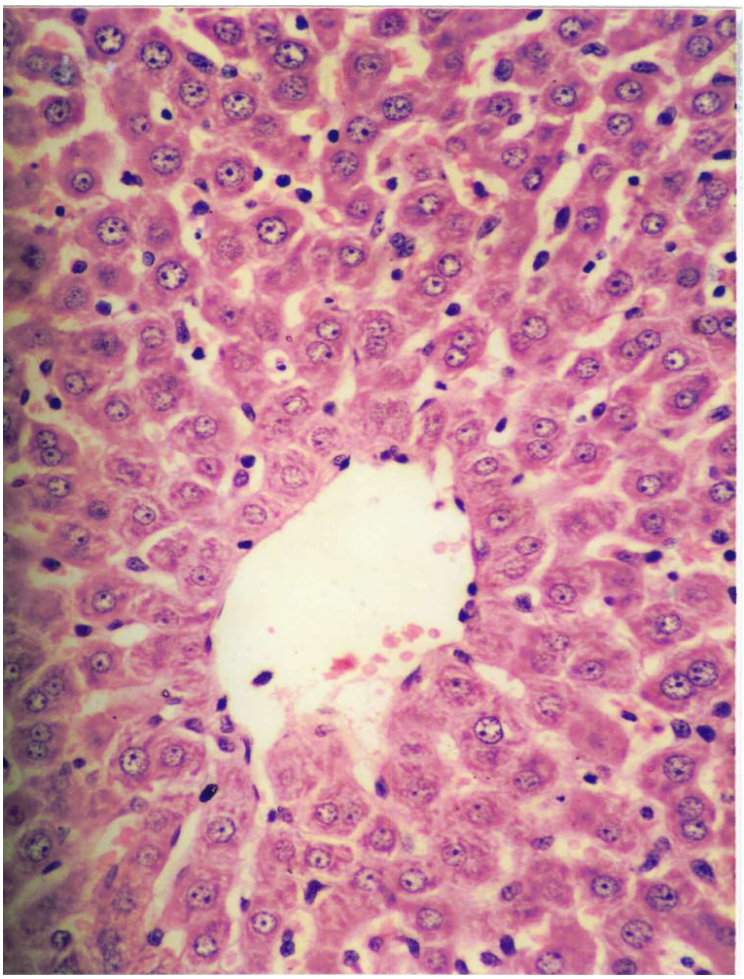


Fig.- 2 (CCl_4 Control group)

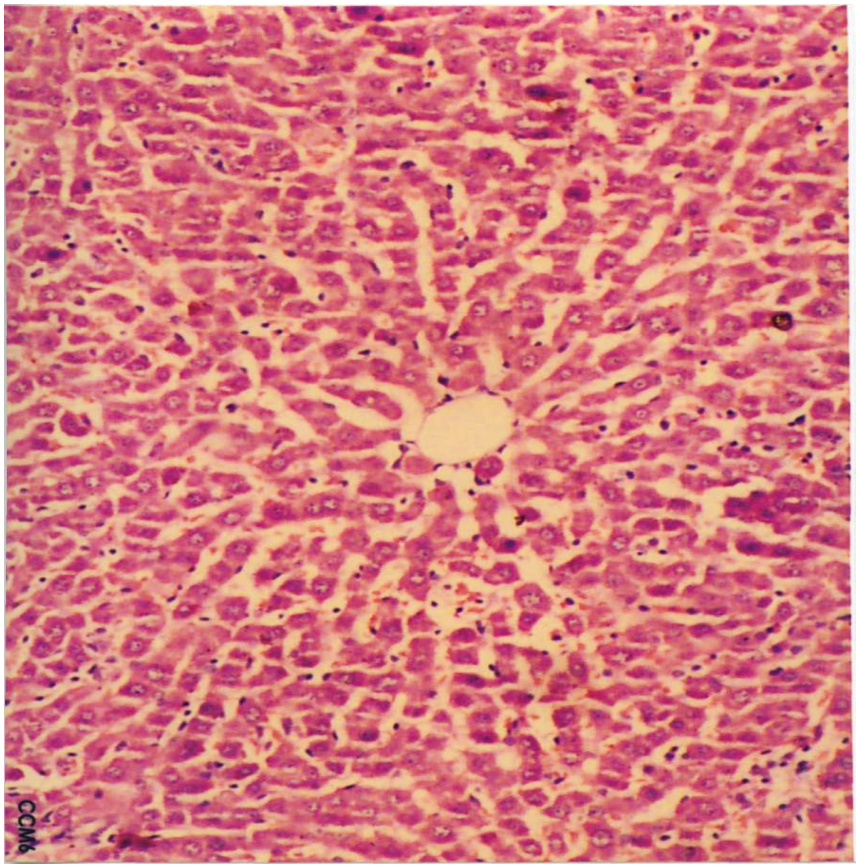


Fig.- 3(CCl_4 Recovery group)

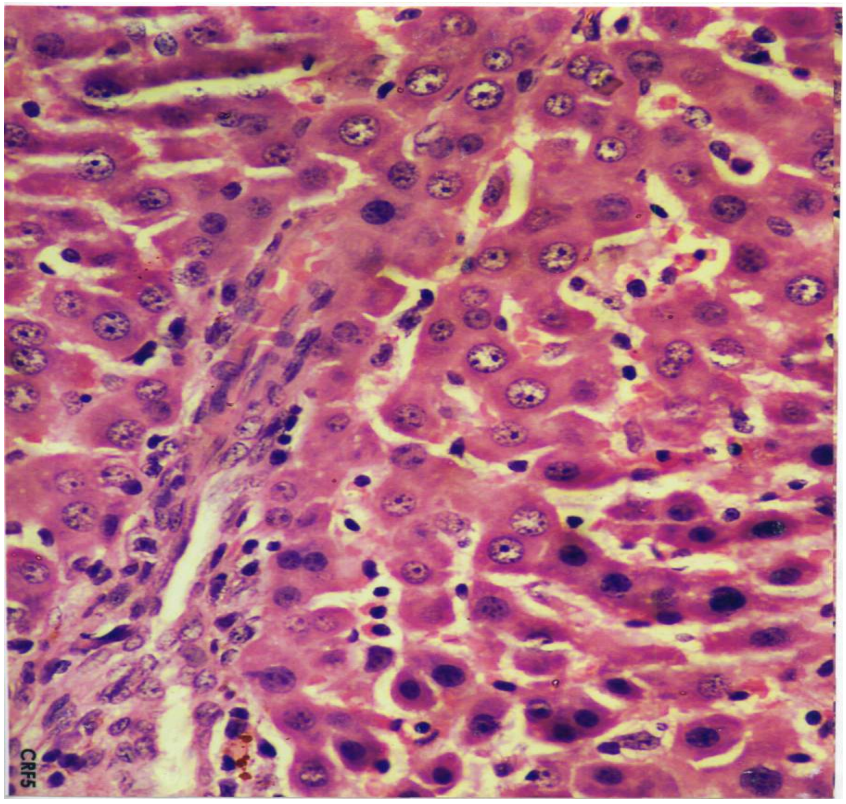


Fig.- 4 (Leaves powder control group)

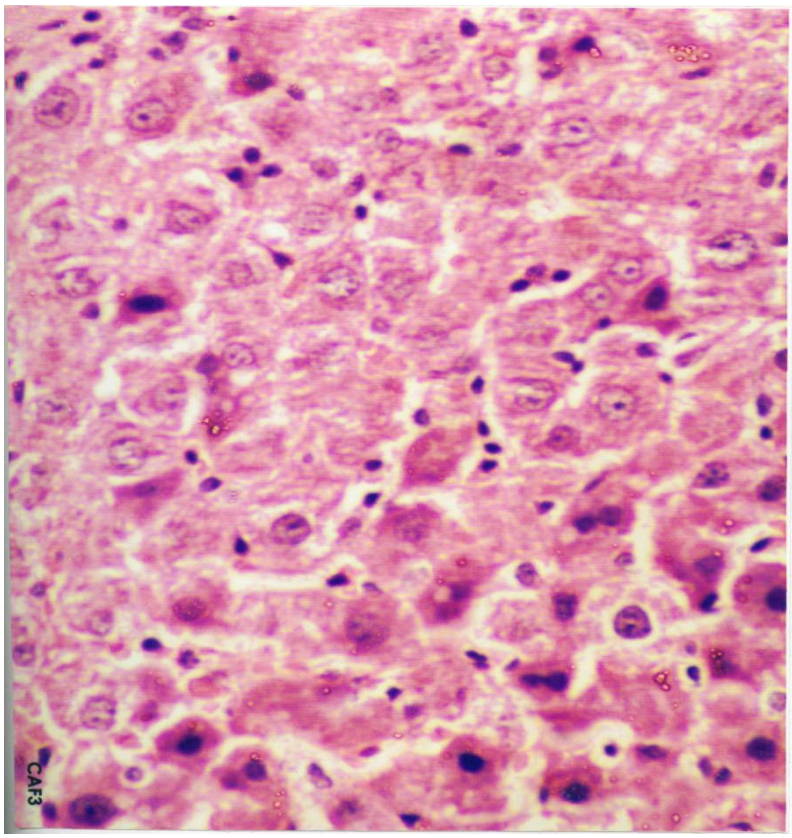


Fig.- 5 (Silymarin control group)

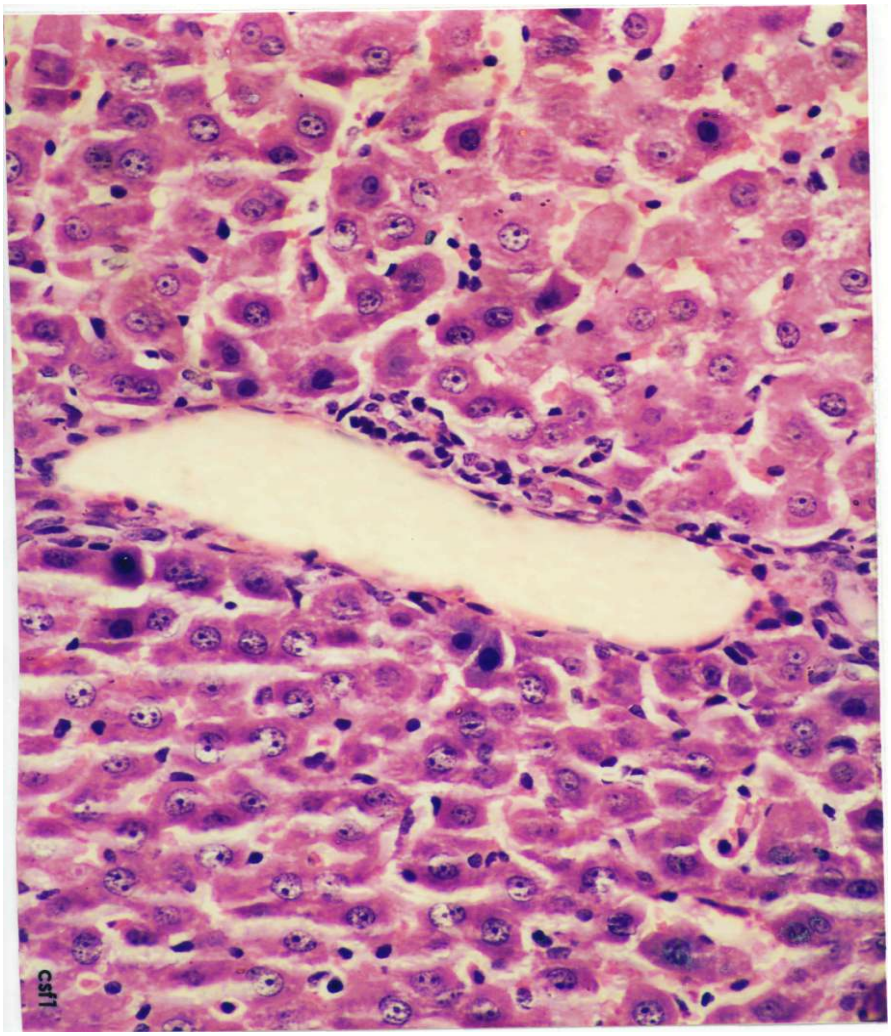


Fig.- 6 (Normal Control group)

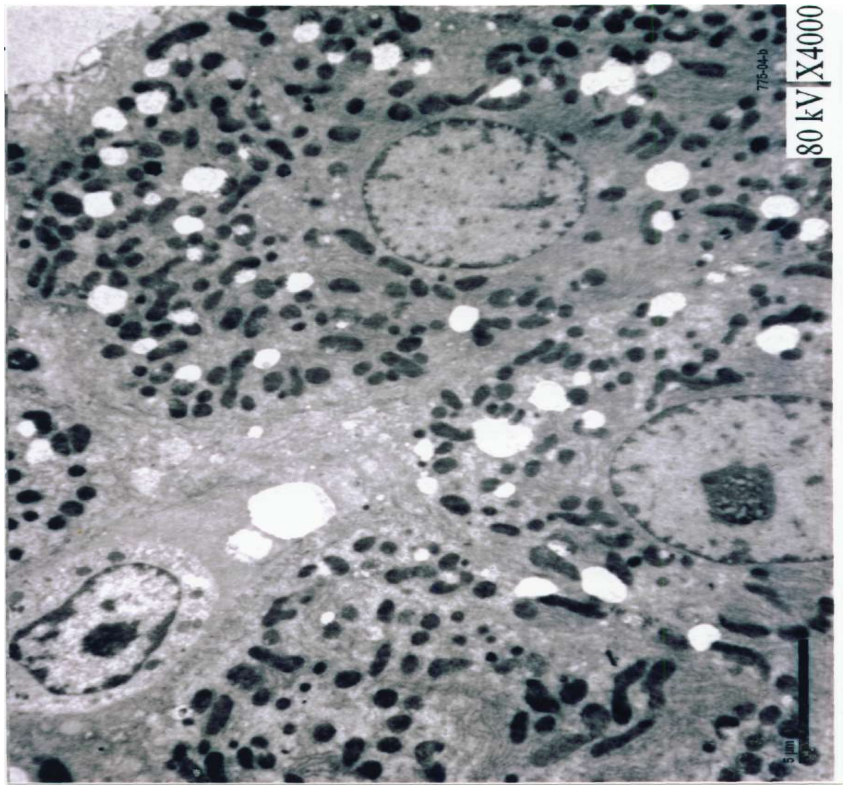


Fig.- 7 (CCl_4 Control group)

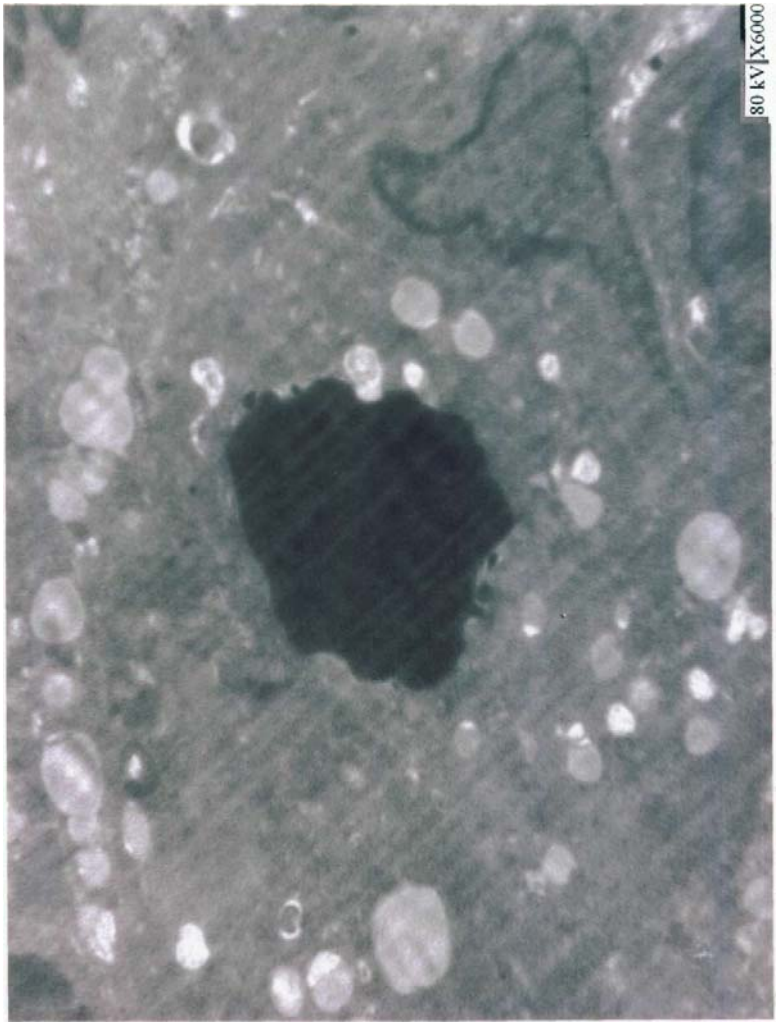


Fig.- 8(CCl_4 Recovery group)

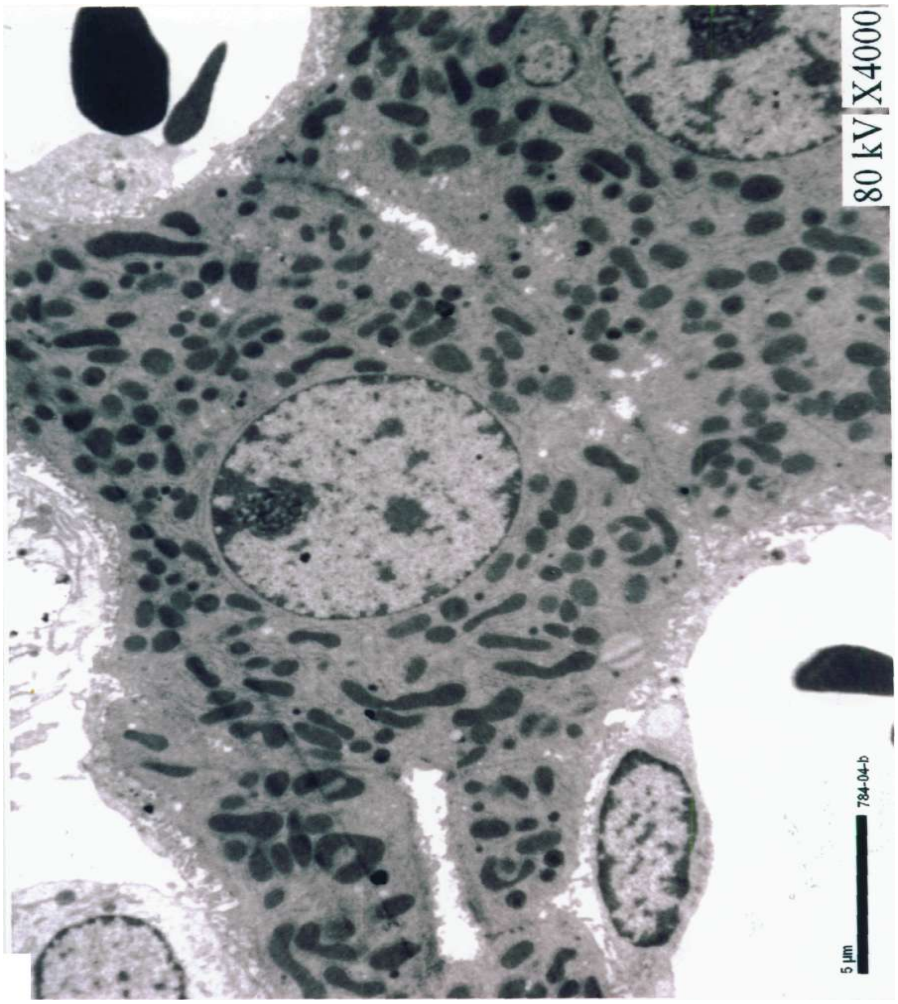


Fig.- 9 (Leaves powder control group)

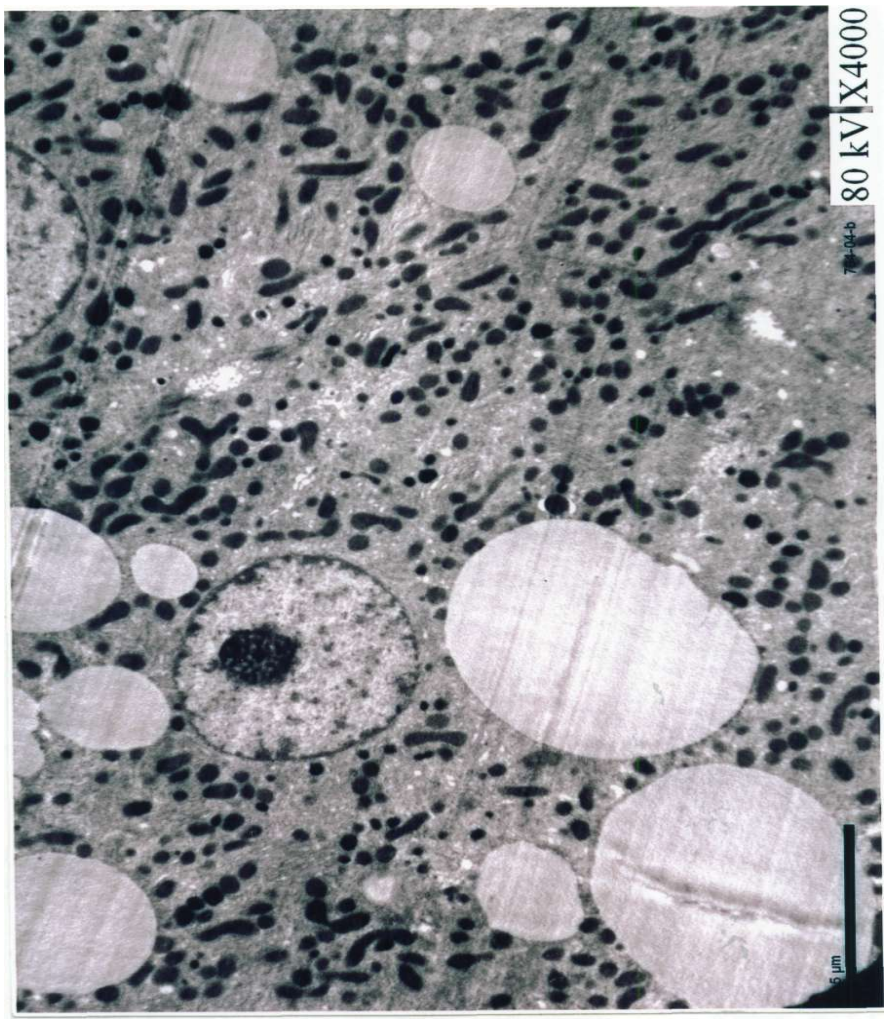
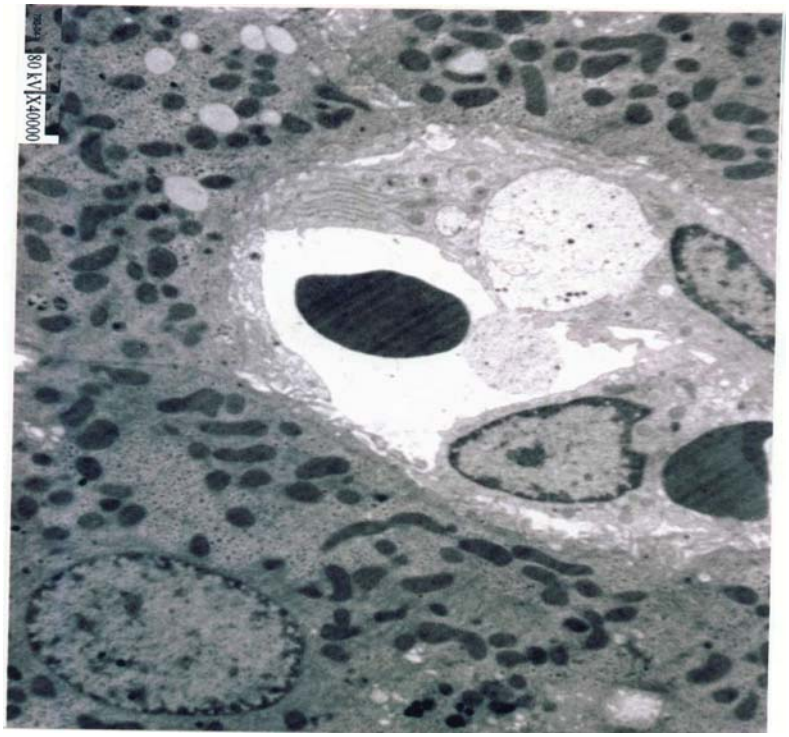


Fig.-10 (Silymarin control group)



Discussion

Carbon tetrachloride is one of the most commonly used hepatotoxin. It is well documented that carbontetrachloride is biotransformed under the action of cytochrome P-450 in the microsomal compartment of liver to trichlomethyl radical which readily reacts with molecular oxygen to form trichloromethyloeroxy radical [12]. This free radical in the presence of oxygen may cause peroxidation of lipid on target cell resulting in extensive damage [13]. Administration of $CC1_4$ (0.7ml s.c.) to rats produced hepatotoxicity showed by significant increase in the serum levels of GOT, GPT and alkaline phosphate in comparison to control group. Also total protein levels were significantly decreased to 3.29g/dl in CCl_4 control groups from the level of 5.65g/dl in normal control group as shown in the Table 2. Aqueous slurry of leaves powder of *Nyctanthes arbor tristis linn* given at dose 500mg/kg not only prevented the rise in serum level of GOT, GPT, alkaline phosphates but also improved serum lipid profile. The results are well comparable with silymarin (standard drug) treated group [14].

Conclusion

Based on the present findings, it can be concluded that the probable mechanism by which the of *Nyctanthes arbor tristis linn* leaves exerts its protective action against CCl_4-induced hepatocellular metabolic alterations could be by the stimulation of hepatic regeneration through an improved synthesis of proteins, or due to its ability to block the bioactivation of CCl_4 by inhibiting the P 450 2E1 activity and/or its accelerated detoxification and the potential to minimise the deleterious effects of free radicals including the peroxy radicals and its antioxidant activity in combination with the inhibition of lipid peroxidation, thereby the of *Nyctanthes arbor tristis linn* leaves can be ranked as hepatoprotective agent by the combined synergistic effect of its constituents and micronutrients rather than to any single factor through free radicals scavenging activity. Further work is going on to isolate the active components, which are responsible for hepatoprotection

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