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Protective effect of *Terminalia Arjuna* against CCl_4 induced liver damage in rats

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ABSTRACT

Carbon tetrachloride (CCl_4) is a well-known hepatotoxin and exposure to this chemical is known to induce liver injury by free radical formation. The present study has been Terminalia arjuna conducted to evaluate the protective role of the aqueous slurry of the bark powder of Terminalia arjuna on CCl_4 induced liver dysfunction. Animals were treated with the aqueous slurry of, (2g/kg body weight) for four days after reversible liver damage induced by administering a single dose of CCl_4 (1ml/kg body weight) in liquid paraffin. Serum marker enzymes were estimated for all study groups. Antioxidant status in the liver tissues was estimated by determining the activities of these enzyme markers; as well as by light and electron microscopic study. Results showed that CCl_4 caused a marked rise in serum marker levels. The aqueous slurry of the bark powder of Terminalia arjuna, successfully prevented the alterations of these effects in the experimental animals and protect the liver tissues against CCl_4 induced oxidative stress probably by increasing antioxidative defense activities.

Keywords: *Terminalia arjuna*, Carbon tetrachloride, hepatotoxin, enzyme markers.

INTRODUCTION

Exposure to various organic compounds including a number of environmental pollutants and drugs can cause cellular damages through metabolic activation of such compounds to highly reactive substances such as reactive oxygen species. Free radical induced lipid peroxidation is believed to be one of the major causes of cell membrane damage leading to a number of pathological situations [1, 2, 3]. Carbon tetrachloride (CCl_4) is a well known potent environmental hepatotoxin [4, 5, 6, 7]. Extensive evidence demonstrated that .CCl_3 and .Cl are formed as a result of the metabolic activation of CCl_4, which in turn, initiate lipid peroxidation process. Some herbal formulations could protect from CCl_4 induced hepatic damage by altering the levels of increased lipid peroxidation, and enhancing the decreased activities of antioxidant enzymes as well as enhanced the decreased level of the hepatic reduced glutathione [8, 9]. India is well known for a plethora of medicinal plants. The traditional Indian medicinal plants act as

antiradicals and DNA cleavage protectors [10]. These plants have also been considered to protect health, longevity, intelligence, immunosurveillance and body resistance against different infections and diseases. *Tephrosia purpurea* [11], *Silybum marianum* [12], *Picrorhiza kurroa* [13], *Cajanus indicus* [14, 15], *Phyllanthus niruri* [16, 17, 18], *Centella asiatica*[19], *Ocimum sanctum*[20], *Adhatoda vasica*[21], *Ricinus communis*[22], *Curcuma longa*[23], *Azadirchta indica* [24], *Tinospora Cordifolia*[25] etc. posses hepatoprotective property against different toxins and drugs induced hepatic disorders.

In this study, protective role of aqueous slurry of the bark powder of *Terminalia arjuna* is evaluated against CCl_4 induced damage in liver. The effects were evaluated by measuring the levels of the serum marker enzymes followed by liver histology.

MATERIALS AND METHODS

Plant Material

The bark of *Terminalia arjuna* was collected from AVSARI FOREST PARK, Ambegaon, Pune, Maharastra, India. After collection of the required quantity of plant material, it was carefully segregated, cut down into small pieces and dried in shade to a constant weight. The plant material was kept in preset oven for a week at 40°C and powdered in high speed electronic mixer and sieved through a BSS Mesh No. 85 sieve and stored in an airtight container with al specifications like date of collection, weight, humidity etc. This plant material was used for animal trials.

Acute Toxicity Study

Acute toxicity studies were carried out for bark powder of *Terminalia arjuna* per OECD guideline in Swiss mice weighing 25 to 30 gm by administering a dose 2, 4 and 6gm/kg body weight orally in the form of aqueous slurry. The groups were almost continuously observed for mortality and behavioral changes during first 24 hr and then daily for a fortnight. There was no abnormality observed in any of these three groups.

Table 1: Daily Doses Regime

DAYS	Group I Normal control	Group II CCl_4. control	Group III CCl_4 treated natural recovery	Group lV CCl_4 + plant material treated	GroupV Silymarin treated
1	0.5cc liq. Paraffin & 2 cc d/w oral	0.7cc/kg CCl_4 in 0.5cc liq. Paraffin i.p.& 2cc d/w oral	0.7cc/kg CCl_4 in 0.5cc liq. Paraffin i.p. & 2cc d/w oral	0.7cc/kg CCl_4 in 0.5cc liq. Paraffin i.p. & 2gm/kg plant material in 2cc d/w oral	0.7cc/kg CCl_4 in 0.5cc liq. Paraffin i.p., 0.007gm/kg Silymarin in 2cc d/w oral
2	2cc d/w oral	2cc d/w oral	2cc d/w oral	2gm/kg plant material in 2cc d/w oral	0.007gm/kg Silymarin in 2cc d/w oral
3	2cc d/w oral	2cc d/w oral	2cc d/w oral	2 gm/kg plant material in 2cc d/w oral	0.007gm/kg Silymarin in 2cc d/w oral
4	Sacrifice	Sacrifice	2cc d/w oral	Sacrifice	Sacrifice
5	-	-	2cc d/w oral	-	-
6	-	-	2cc d/w oral	-	-
7	-	-	Sacrifice	-	-

All dosages are for each individual animal in the group. The number of animals in each group 12 (6 males + 6 females).
i.p. : intraperitoneal.
d/w : Distilled Water.

Gr. I served as Normal Control; Gr. II served as CCl_4 Control, Gr. III served as CCl_4 Recovery, Gr. IV served as CCl_4 + bark powder of *Terminalia arjuna* in the form of aqueous slurry and Gr. V served as CCl_4+ Silymarin (a known hepatoprotectant).

Animals

Wistar albino rats (120-150 g) of either sex were used for this study. The animals were maintained under standard laboratory conditions at temperature 25±2^{0}C with relative humidity 20±5 % and 12h light and dark cycle throughout all the experiments. Animals had free access to food and water *ad libitum*. The dose selected for bark powder of *Terminalia arjuna* in the form of aqueous slurry is 2g/Kg body weight against CCl_4 damaged liver in rats. Animals were grouped into five groups. Each group consists of 12 animals, 6 males and 6 females. Reversible liver damage was induced by 0.7ml/Kg of CCl_4 in 0.5 ml. Liquid Paraffin per animal i.p. The dose of plant powder in the form of aqueous slurry was given orally via gavages as per dose chart in Table 1.

Assessment of liver function

The animals from all groups were sacrificed on IVth day and for of the study except the natural recovery group which was sacrificed on VIIth day after natural recovery of liver was initiated. The blood sample was collected by cardiac puncture, blood and tissue biochemical examinations were carried out to assess liver function by using standard diagnostic kits.

Assessment of liver function

A single dose of CCl_4 resulted in a significant change in serum AST, ALT, Alkaline Phosphate, bilirubin, total protein+ levels etc. The treatment with bark powder of *Terminalia arjuna* in the form of aqueous slurry exhibited an ability to counter act the CCl_4 induced hepatotoxicity by regaining the AST, ALT, Alkaline Phosphate and bilirubin levels like normal rats. Histopathology of liver of the normal control rats showed prominent central vein and normal arrangement of hepatic cells (Fig.I). CCl_4 treated rats showed various degrees of pathological changes starting from centrilobular necrosis of hepatic cells to central lobular fatty degeneration (Fig. II). The natural recovery group shows some initial signs of recovery indicating initiation of slight recovery (Fig III). Liver photographs of the rats treated with bark powder of *Terminalia arjuna* in the form of aqueous slurry showed significant regeneration against CCl_4 induced liver damage (Fig IV). The sections of liver taken from the rats treated with standard drug Silymarin showed the hepatic architecture less recovered than the plant treated group(Fig. V).

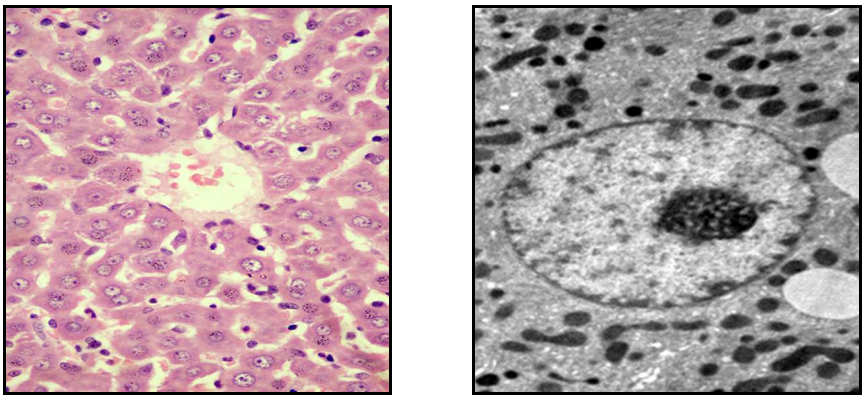

Fig. I: Light and Electron micrograph of normal rat liver

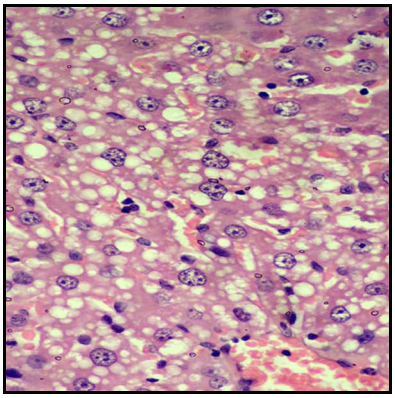
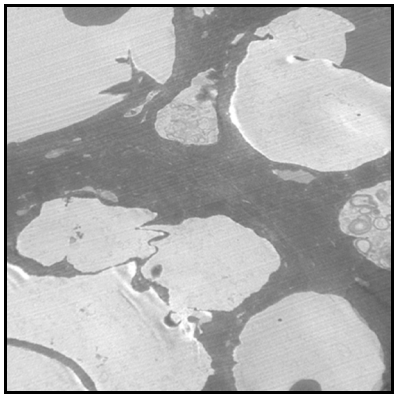

Fig. II:Light and Electron micrograph of rat liver after CCl_4 treatment

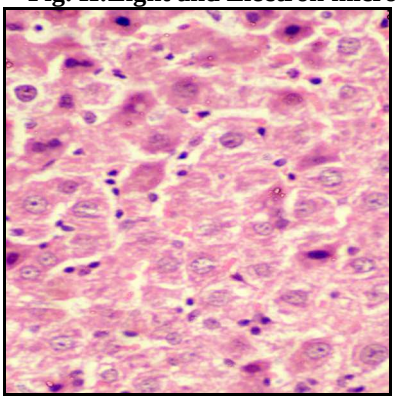
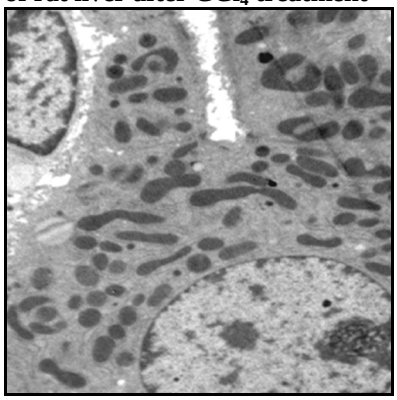

Fig. III: Light and Electron micrograph of rat liver after Natural Recovery

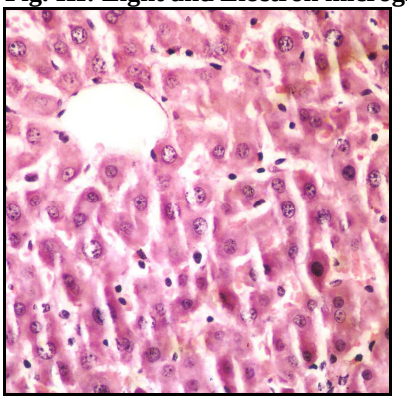
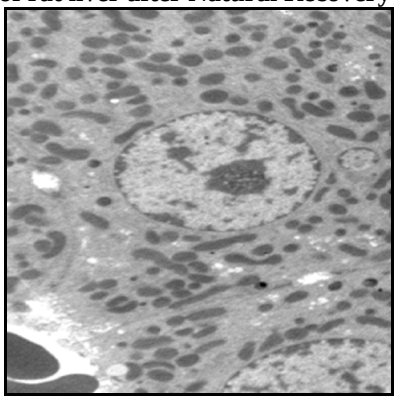

Fig. IV: Light and Electron micrograph of rat liver treated with CCl_4 and plant material

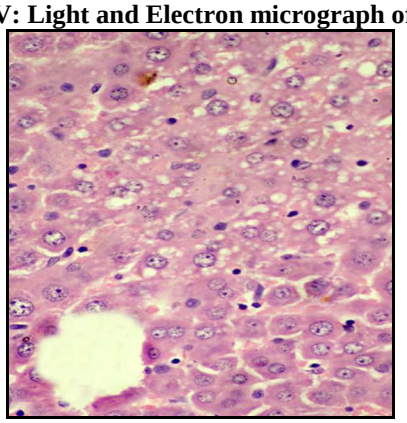
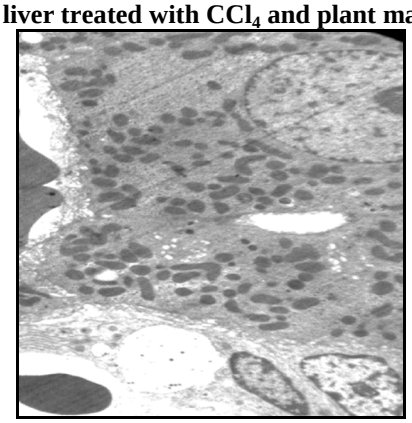

Fig. V: Light and Electron micrograph of rat liver treated with CCl_4 and Silymarin

DISCUSSION

Liver has an important place in qualitative and quantitative toxicology by virtue of its function. The single dose of CCl_4 has been used as a tool to induce hepatotoxicity in experimental animals. CCl_4 induced liver damage is due to its cytochrome P-450 enzyme system catalyzed hepatic conversion into highly reactive trichloromethyl radical (CCl_3), which upon reaction with oxygen radical gives trichloromethyl peroxide radical ($OOCCl_3$). The radical form a covalent bond with sulfhydryl group of several membrane molecules like glutathione, considered as the initial step in the chain of events leading to lipid peroxidation and liver tissue destruction. Normal liver functions are characterized by the balanced activities of serum marker enzymes AST, ALT, Alkaline Phosphate, bilirubin as well as total protein. Hepatocellular necrosis leads to very high level of AST and ALT released from liver in the blood. Among the two, ALT is a better index of liver injury, as liver ALT activity represents 90% of total enzyme present in the body. Alkaline phosphate activities on the other hand are related to the functioning of the hepatocytes, increase in its activity is due to increased synthesis in presence of increased biliary pressure.

The bark powder of *Terminalia arjuna* if given in the form of aqueous slurry decreases the elevated enzyme levels of AST and ALT, which suggests the protection of structural integrity of hepatocyte cell membrane or regeneration of damaged liver cells by the plant material caused by carbon tetrachloride. This effect is an agreement with the view that serum marker levels returned to normal with healing of hepatic parenchyma and regeneration of hepatocytes.

The observations of blood and tissue Biochemical Parameters for all Groups were given in table 2.

Table 2: Tissue Biochemical Parameters of all Groups

Parameter	Gr.I	Gr.II	Gr.III	Gr.IV	Gr.V
Gycogen	20.5±1.2	20.40±1.3	22.30±1.2	19.60±1.2	17.5±1.4
T. Protein	4.4±1.0	20.2±1.1	10.5±1.3	6.1±1.2	9.1±1.3
Cholesterol	1.6±0.4	2.30±0.5	1.90±0.5	1.8±0.6	2.8±0.7
DNA	0.5±0.01	0.45±0.12	0.90±0.14	0.48±0.20	0.7±0.13
RNA	2.4±0.1	4.9±0.1	3.75±0.2	2.3±0.1	6.5±0.3

Table 3: Blood Biochemical Parameters of all Groups

Parameter	Gr.I	Gr.II	Gr.III	Gr.IV	Gr.V
GPT(ALT)	55.60±1.1	50.08±1.1	41.20±1.2	54.21±2.0	66.50±1.1
GOT(AST)	44.00±1.0	46.20±1.4	48.30±1.3	47.20±1.4	56.84±1.2
Cholesterol	75.60±1.3	82.40±1.2	75.40±1.2	76.70±1.4	69.50±1.4
Bilirubin	0.58±0.2	0.68±0.3	0.64±0.3	0.6 ±0.2	0.65±0.2
Triglecerides	124.50±1.2	130.00±2.1	94.80±2.1	120.20±1.8	124.50±2.3
√GT	18.30±1.0	41.20±1.5	33.40±1.3	21.50±1.2	24.80±1.2

However considerable increase in total proteins supports the normal function of the liver. The bark powder of *Terminalia arjuna* in the form of aqueous slurry exerts a clear-cut protective action against carbon tetrachloride induced hepatic damage in rats.

CONCLUSIONS

The present work is carried out to investigate the hepatoprotective action of the bark powder of *Terminalia arjuna* in the form of aqueous slurry on CCl_4 induced liver damage in rats. Blood biochemical assays like GPT(ALT), GOT(AST), Cholesterol, Bilirubin, Triglecerides and √GT and tissue biochemical assays like Gycogen, T. Protein, Cholesterol, DNA, and RNA have been studied for evaluation of the same. From the examination of these parameters it is demonstrated that the bark powder of *Terminalia arjuna* in the form of aqueous slurry gave best recovery for CCl_4 induced hepatic injury. The observations of "Group I" were matching with "Group IV" than all other groups. The combined synergistic effect of its constituents and micronutrients rather than to any single factor through free radicals scavenging activity play important role in regeneration of liver cells.

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