Full Length Research Paper

Hepatoprotective and hepatocurative properties of alcoholic extract of *Carthamus oxyacantha* seeds

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Carthamus oxyacantha commonly known as "*Poli*" or "*Peeli kandiary*" belongs to family *Asteraceae/Compositae*. Protective and curative effects of alcoholic extract of *C. oxyacantha* seeds were investigated against carbon tetrachloride (CCl_4) induced hepatic damage in rats (Sprague Dawley Strain). Hepatic damage was induced by injecting a single intraperitoneal dose of 4 ml (50%v/v) CCl_4 in olive oil. In protective studies, plant extract (400 mg/kg body weight) was given before hepatic damage while in curative studies; hepatic damage was induced before the application of plant extract. The hepatoprotective and hepatocurative effects were monitored by estimating the activities of the serum glutamate pyruvate transaminase (SGPT), serum glutamate oxalacetate transaminase (SGOT), serum alkaline phosphatase (ALP), total proteins, glutathione level serum bilirubin concentration and histopathological studies. Results show that alcoholic extract of seeds of *C. oxycantha* possessed both hepatoprotective and hepatocurative activity. However, hepatoprotective activity was more pronounced as compared to hepatocurative activity. Histopathological studies also supported the biochemical parameters.

Key words: *Carthamus oxyacantha,* hepatoprotective, hepatocurative.

INTRODUCTION

Hepatotoxicity is inflammation of the liver. It is generally associated with various drugs used in modern medicine, different chemicals, toxins and viruses (Ravikumar et al., 2005; Stierum et al., 2005). Hepatic problems along with heart problems and diabetes are one of the major causes of human death across the world. Roger et al. (2001) reported that more than two million people die annually from liver related disorders in the world.

One of the known hepatotoxins used in biological research is Carbon tetrachloride (CCl_4). Inside the body, CCl_4 produces trichloromethyl free radicals. These free radicals react with other molecules in the cell and stimulate a series of reactions. End result of this series of reactions is the initiation of peroxidation of membrane lipids (Reinke et al., 1988; Sahu et al., 2005) and hence

liver damage. Trichloromethyl radical is believed to be an intermediate product of the reductive dechlorination of CCl_4, catalyzed by certain cytochrome P_{450} enzymes particularly the ethanol inducible isoform of the cytochrome (Reinke et al., 1988). There are many reports which show similarities between CCl_4 induced liver damage and human liver cirrhosis (Halim et al., 1997). That is why CCl_4 induced liver damage is generally used as experimental model for screening of hepatoprotective and hepatocurative drugs. The intensity of hepatic damage is generally accessed by measuring the activities of hepatic cytoplasmic enzymes [serum glutamate pyruvate transaminase (SGPT), serum glutamate oxalacetate transaminase (SGOT), serum alkaline phosphatase (ALP)], serum bilirubin concentration and

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histological studies (Ravikumar et al., 2005; Stierum, 2005). The extent of oxidative stress may be predicted by estimating the serum glutathione level (Sallie et al., 1991; Sahu et al., 2005).

Whereas no reliable cure is available for hepatic disorders in modern medicine practice, herbal treatment has recommended variety of plants for its treatment (Sanmugapriya and Venkataraman, 2006). The efficacies of most of these medicinal plants have not yet been validated. Scientific-based pharmacological data is not available for most of the herbal formulations. Hence, all such herbal formulations cannot be recommended for liver diseases (Stickel and Schuppan, 2007). Thus, investigation of medicinal plants with potential hepatic regenerative activity becomes important.

Carthamus oxyacantha is commonly known as *Poli* or *Peeli Kandiari* in different parts of the Pakistan. It is an annual herb with spiny leaves. Like other spiny plants in the genus *Carthamus*, this species is not used as fodder for livestock.

Plants in genus *Carthamus* are reported to have a mixture of glycerides composed of linoleic and oleic acids. Concentration of these compounds varies in different species. San Feliciana et al. (1982) reported the presence of a sesquiterpene glycoside as the dominant component in hexane extract from aerial parts of *C. lanatus.* This compound seems to be characteristic of genus *Carthamus.* Many plants in family *Asteracceae / Compositae* are reported to contain pyrrolizidine alkaloids which are reported to be hepatotoxic (Mattocks, 1990; Borba et al., 2001). Pyrrolizidine alkaloids are a diverse class of natural compounds based on azabicyclo ring, generally occurring as esters of a "necine base" with "necic acids" as mono- or diesters (Mattocks, 1990).

The seeds of *C. oxyacantha* are used by many local *Tabibs* for treatment of diabetes in some parts of the Pakistan. However, scientific data is still not available about the physiological effects of this plant. We hope that this study will help us to evaluate the exact use of this plant in treatment of different diseases.

MATERIALS AND METHODS

Plant collection

C. oxyacantha seeds were collected from fields of different parts of District Attock, Chakwal, Jehlum and Rawalpindi. Plant and it seeds were then brought to the Department of Biochemistry, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad. Plant was identified at the Department of Plant Sciences (voucher no. 109, dated; 21-8-2006), Faculty of Biological Sciences, Quaid-i-Azam University Islamabad. The seeds were then thoroughly washed with distilled water and dried in an oven at 30°C.

Preparation of plant extract

The dried seeds were powdered mechanically with a China herb grinder. The powder was kept in dry, clean, air tight glass jars and lipids and was filtered. The residue was mixed with 250 ml absolute ethanol (Merck) for 48 h with occasional shaking and was filtered. The filtrate was dried in Petri dishes and concentrated to greenish / brownish residue (4.6±0.07 g/100 g plant material) by evaporation at 4°C under reduced pressure in vacuum drying oven (Toyo Seisakusho co, Japan). The dried alcoholic extract was stored in a refrigerator until used. The extract was dissolved in adequate amount of 50% ethanol just before injection to respective group of rats. The extract was given as intraperitoneal injection as desired.

Experimental animals

Healthy young adult male albino rats of Sprague Dawley strain, weighing between 180-300 g were obtained from the National Institute of Health, Islamabad and kept at the animal house, Department of Biochemistry, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad. The animals were kept on standard feed purchased from National Institute of Health, Islamabad. The animals were handled according to European Community guidelines (EEC Directive of 1986; 86/609/EEC).

Grouping

25 rats were randomly divided into five groups of five rats each. All rats were maintained on standard feed and water *ad libitum* with natural day light and dark periods. Group 1 was normal control and Group 2 was sham control for hepatoprotective studies. Animals in this group were given a single dose of CCl_4 (50% v/v in olive oil) on the 30th day to cause hepatic damage. Group 3 was sham control for hepatocurative studies. Animals in this group were given a single dose of CCl_4 (50% v/v in olive oil) on the 1st day to cause hepatic damage. Rests of the two groups (4 and 5) were treated. Animals in group 4 were given plant extract 400 mg/kg body weight for 29 days and a single dose of CCl_4 (50% v/v in olive oil) on the 30th day. Animals in group 5 were given a single dose of CCl_4 (50% v/v in olive oil) on the 1st day and thereafter plant extract 400 mg/kg body weight for 30 days. All rats were sacrificed on the 31st day.

Blood collection

Animals were anaesthetized on the 30th day and heart puncture was done with a 5-ml disposable syringe. 2 ml blood was drawn very gently and slowly. Serum was separated by centrifugation at 3000 rpm for 15 minand then preserved in the appendorf tubes at -20°C in the freezer until analyzed.

Serum analysis

Serum samples collected from different groups were analyzed for chemical estimation by using packed kits made by Sigma (USA). The absorption was recorded using a Spectrophotometer UV-240 (Shimadzu). All estimations were made according to the procedure provided with the kit. All values given are mean ±SE of 5. Computer program "Statistica 5.5" was used for statistical analysis. Student's t-test" and "ANOVA" were applied to find the difference between normal control, sham control and treated groups. A "p" value of 0.01 or < 0.01 was taken as a level of significance.

Histological studies

Lives were removed in respective groups and stored immediately in a solution containing 10% formalin and 0.9% NaCl for histopatholo-

Table 1. Effect of *C. oxyacantha* seeds extract on body weight and liver weight in hepatoprotective and hepatocurative studies.

Group	Initial body weight (g)	Final body weight (g)	Difference in body weight (g)	Body weight gain/loss (%)	Weight of liver (g)	Liver/body weight (%)
Hepatoprotective studies						
Normal control	180.1±10.3	191.3±12.2	11.1±3.7	6.16±1.8[a]	8.4±0.23	4.4±0.4[a]
Sham control	199.3±14.5	212.4±15.4	13.1±2.9	6.5±1.4[a]	7.2±0.45	3.89±0.6[a]
Treated	179.2±16.8	190.9±13.1	11.7±3.2	6.5±1.4[a]	6.4±0.14	3.35±0.2[a]
Hepatocurative studies						
Sham control	220.8±17.2	211.7±14.3	-9.1±3.1	-4.12 ±0.9[b]	5.2±0.31	2.5±0.8[b]
Treated	187.4±20.5	183.8±18.3	-3.6±0.6	-1.92±0.06[c]	5.8±0.2	3.2±1.1[b]

Values given are mean ±SE of 5. Sham control was compared with normal control. The treated was compared with respective sham control. Values with same superscript in a column differ non-significantly while with different superscript differ significantly at p<0.01.

gical examination. The tissues were then embedded in paraffin, thinly sectioned using a microtome, stained with haematoxylin and eosin (H&E) and examined under optical microscope.

RESULTS AND DISCUSSION

Hepatoprotective studies are conducted to investigate the protective / preventive effects of the plant extracts against liver damage. Plant extract 400 mg/kg body weight was given prior to the liver damage in hepatoprotective studies. The extract at the doses of 250 and 500 mg/kg were comparable to the effect produced by Liv-52®, a well established plants-based hepatoprotective formulation against hepatotoxins (Arulkumaran et al., 2009).

Hepatocurative studies were conducted to investigate the efficacy of plant extract in curing the liver damage. In these studies, liver damage was induced prior to the application of plant extract. In both the hepatoprotective and hepatocurative studies, liver damage was caused by a single dose of CCl_4.

In hepatoprotective studies, a net body weight gain was observed in all the groups (Table 1) including control (6.16±1.8%), sham control (6.5±1.4%) and treated which is in accordance with previous finding of Smialowicz et al. (1991). However, Shibayama (1989) reported a 6-8% loss in body weight in sham control group. This difference may be due to the difference in experimental designs. In our study, hepatic damage was induced on the 30th day and experiment was run for 31 days.

The percent mean body weight gain in control group (1); sham control group (2) and treated group (4) differ non-significantly. Similarly, no significant difference was observed in percent liver / body weight ratio between control (4.4±0.4%) group and sham control group (3.89±0.6%). This was expected because the experiment was run for one month and liver damage was induced on the second last day (30th). On the very next day (31st day) rats were sacrificed. CCl_4 was not given enough time to

affect the metabolism, body weight and percent liver / body weight ratio. Therefore, no significant difference in mean percent liver / body weight ratio in different groups of rats were found.

In hepatocurative studies, a net body weight gain was observed (Table 1) in the control group (6.16±1.8%) as compared to a net body weight loss (4.12±0.9%) in sham control group (3). In the treated group (5) however, a weight gain was observed (5.8±0.2). A body weight loss in sham control group was expected because CCl_4 was given on the 1st day and rats were sacrificed on the 31st day. A complete one month is quite enough time for CCl_4 to severely affect the body metabolism and hence causing a decrease in body weight. Similarly, a significant difference in percent liver / body weight ratio was observed between control group (4.25±0.4%) and sham control group (2.5±0.8%).

The animals in the sham control groups of both hepatoprotective (on 30th day) and hepatocurative (1st week of the experiment) studies were quite sluggish and less active as compared to the control group. The animals in treated groups of hepatocurative studies showed same symptoms. This may be due to the fact that CCl_4 also acts as an anesthetic agent, causing slowness in all reflexes of the animal and other general behavior.

A significant difference in liver marker enzymes that is ALP, SGPT, SGOT and serum glutathione level was observed between control and sham control groups of both hepatoprotective and hepatocurative studies (Table 2 and Figure 1). This shows the hepatic damage caused by CCl_4.

Smialowicz (1991) had reported no notable change in the ALP value in sham control group while Vadiraja et al. (1998) had reported a 5-8 times increase in SGPT and 10-13 times increase in SGOT value in CCl_4 treated group. In the present study, about 4 times increase in SGPT, 5 fold increase in SGOT and 2 fold increase in ALP was observed in sham control groups compared to normal control group. Similarly, a significant decrease in

Table 2. Effect of *C. oxyacantha* seeds extracts on liver marker enzyme activities, bilirubin, glutathione concentration and serum total proteins in hepatoprotective and hepatocurative groups.

Group	ALP (U/L)	SGPT (U/L)	SGOT (U/L)	Bilirubin (mg/dl)	Glutathione (mg/dl)	Total protein (g/dl)
Hepatoprotective studies						
Normal control	112.3±10.6[a]	36.2±6.9[a]	38.1±5.8[a]	0.48±0.03[a]	30.4±6.2[a]	7.3±0.4[a]
Sham control	240.4±16.3[b]	163.8±11.5[b]	186.7±12.2[b]	0.49±0.07[a]	23.3± 4.6[b]	7.1±0.7[a]
Treated	140.4±10.7[c]	87.51±6.4[c]	69.78±7.3[c]	0.3±0.05[a]	29.3±4.8[c]	7.2±0.05[a]
Hepatocurative studies						
Sham control	261.4±16.2[b]	177.6±12.7[b]	176.4±14.7[b]	1.3±0.07[b]	14.7±3.7[b]	4.1±0.3[b]
Treated	140.37±9.5[c]	87.51±11.2[c]	109.78±7.5[c]	0.61±0.03[c]	24.8± 2.5[c]	6.8±0.06[c]

Values given are mean ±SE of 5. Sham control was compared with normal control. The treated was compared with respective sham control. Values with same superscript in a column differ non-significantly while with different superscript differ significantly at p<0.01.
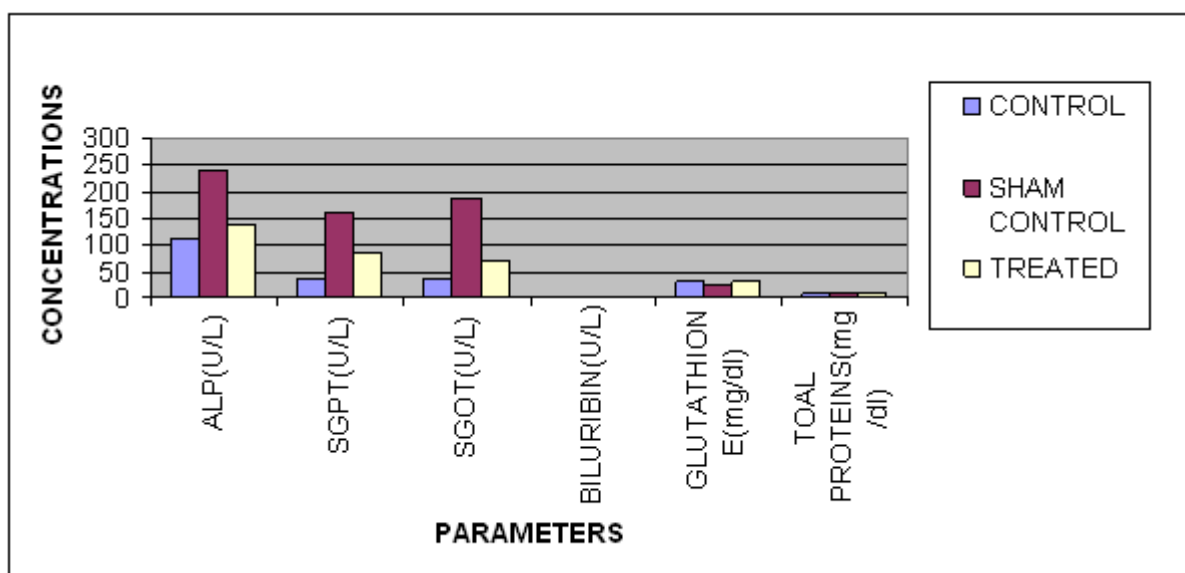
Figure 1. Concentrations of different parameters for different control groups (Hepatoprotective studies).

the serum glutathione level in sham control group (23.3±4.6 mg/dl) was observed as compared to the control group (30.4±6.2 mg/dl) both in hepatoprotective as well hepatocurative studies. In hepatocurative studies, the decrease in glutathione level in sham control group (14.7±3.7 mg/dl) was even more as compared to the control group. This shows an increased use of glutathione which is also characteristic of oxidative stress as a result of hepatic damage due to the CCl$_4$.

In hepatoprotective studies, no significant difference in the value of serum bilirubin and serum total proteins was observed between control group, sham control group and the treated group (Table 2 Figure 1). This might be due to the fact that CCl$_4$ could not get enough time (24 h) to effect the bilirubin concentration and serum total proteins level. Perhaps to affect the bilirubin and total proteins

more time is required by the CCl$_4$. However, in hepatocurative studies, a significant difference in bilirubin and total proteins level was observed between control group and sham control group.

Histological studies of the liver also showed severe damage to the hepatocytes. Necrosis of the hepatocytes is quite prominent in rats in sham control group of both hepatoprotective as well as hepatocurative group (Figures 4 and 5) as compared to the control group (Figure 3).

Alcoholic extract of seeds also showed significant hepatoprotective and hepatocurative effects. A significant decrease in liver marker enzymes, bilirubin and a significant increase in glutathione and serum total proteins was observed in treated groups as compared to the sham control group (Table 2 and Figure 2).
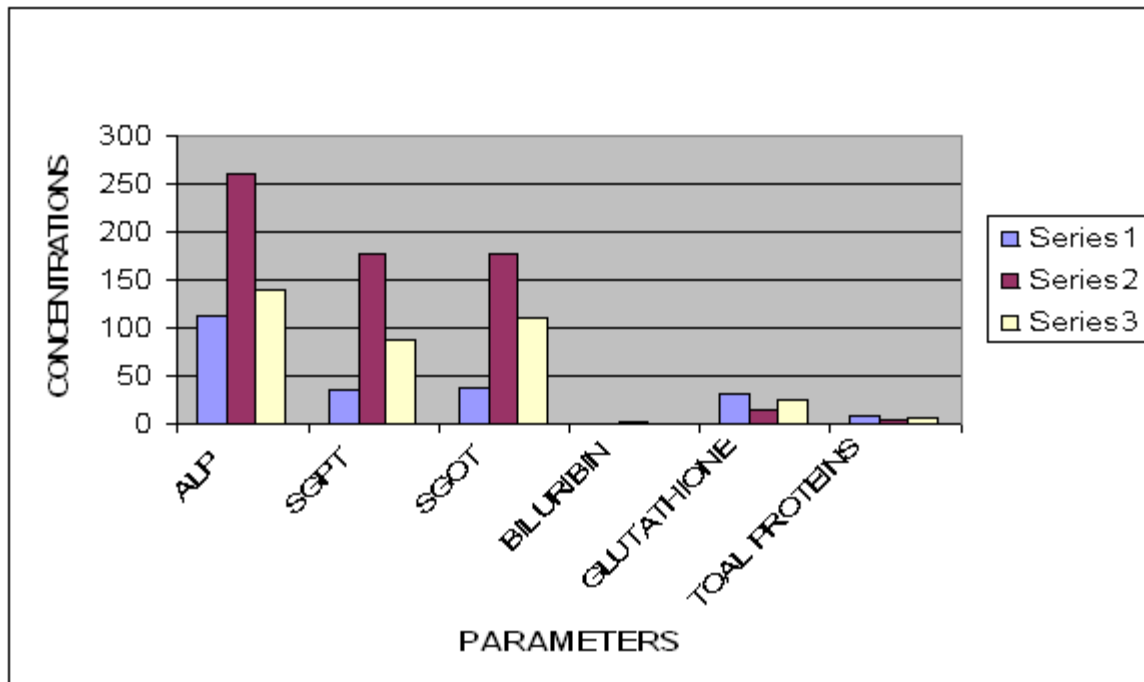
Figure 2. Concentrations of different parameters for different control groups (hepatocurative studies).
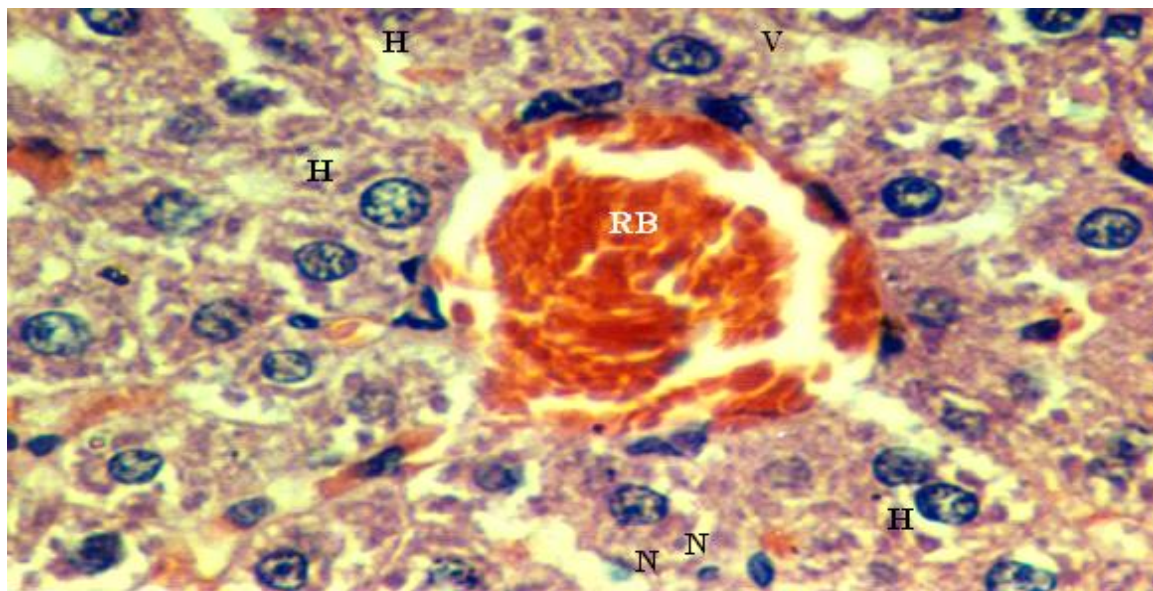

Figure 3. Photograph of the liver of a normal rat at 40X showing normal hepatocytes (H) with prominent nuclei (N), red blood cell (RB) and central vein (CV) (hepatoprotective studies).

Histological studies also show less damage in treated groups as compared to the sham control group (Figures 6 and 7). This reflects the presence of chemicals / compounds like antioxidants which help the hepatocytes to get cured from the damage. *Carthamus tinctorius,* is reported to be having a mixture of glycerides of linoleic and oleic acids in proportions that differ with plant variety.

San Feliciana et al. (1982) reported that a sesquiterpene glycoside is the major component of the hexane extract from aerial parts of *C. lanatus* and that this type of compound seems to be characteristic of genus *Carthamus.*

CCl_4 can cause damage to many tissues in the body. However, the most important primary target organ for
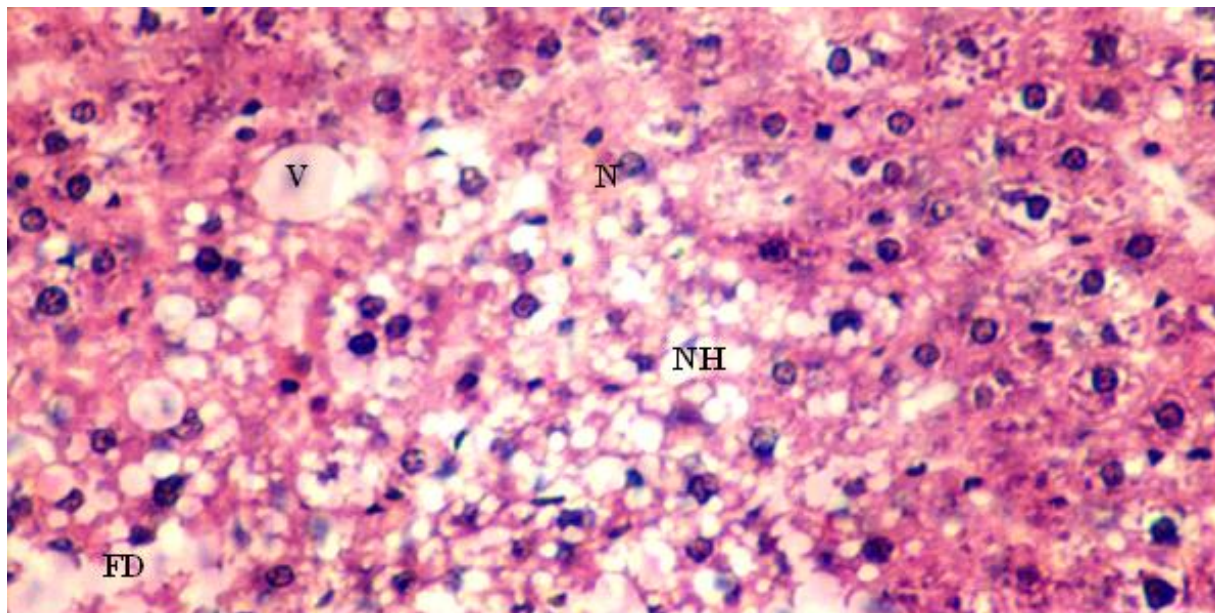
Figure 4. Photograph of the liver of a Sham control (hepatoprotective studies) rat at 40X showing necrotizing hepatocytes (NH) with degenerating nuclei (N), fatty deposition (FD), fibrosis (F) and vacuolization (V).
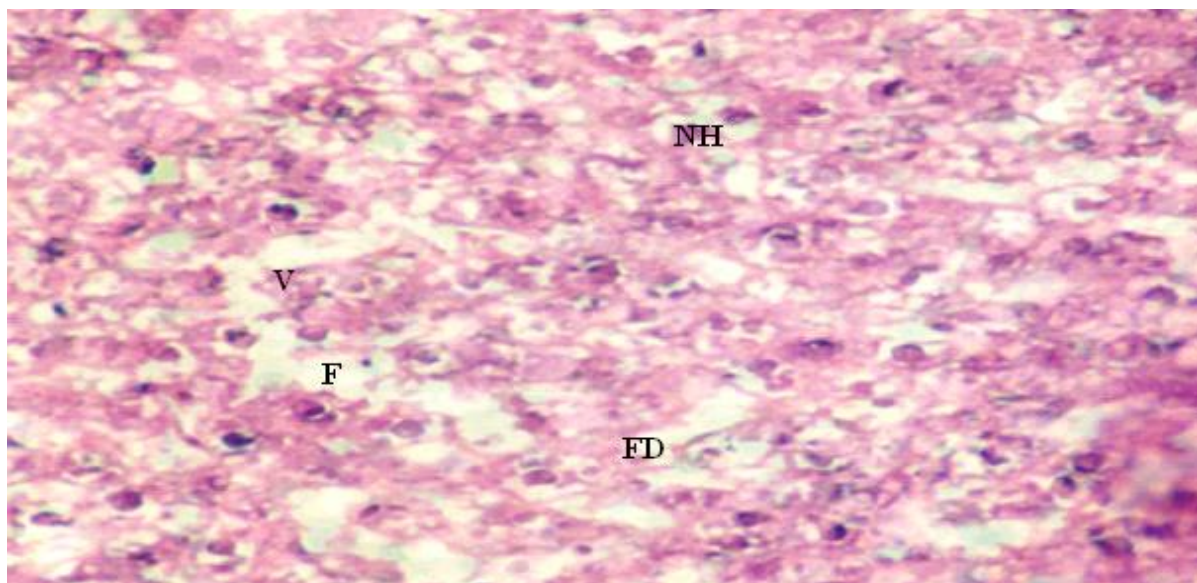

Figure 5. Photograph of the liver of a Sham control (hepatocurative studies) rat at 40X showing necrotizing hepatocytes (NH) fatty deposition (FD), vacuolization (V) and fibrosis (F).

CCl_4 induced toxicity in many species is the liver. CCl_4 when metabolized in the body is changed into a very reactive free redicals (halogenated free radical) by cytochrome P_{450} mixed function oxidase system (Meunier et al., 2004). These reactive species then induce hepatic damage. Many latest evidences show that oxidative stress caused by free radicals may induce peroxidation and damage to biomolecules (lipid protein and nucleic acids). This may further leads to aging, cancer and many other diseases in human. Further it has been observed that inflammatory state of the body (acute or chronic) is due to oxidative stress induced by the generation of free radicals (Van de straat et al., 1987; Sahi et al., 2004). CCl4 also causes severe fatty changes in liver (Afaf et al., 2008).

Hepatoprotective and hepatocurative properties of plants or plants extracts are generally attributed to the presence of chemicals which act as antioxidants or
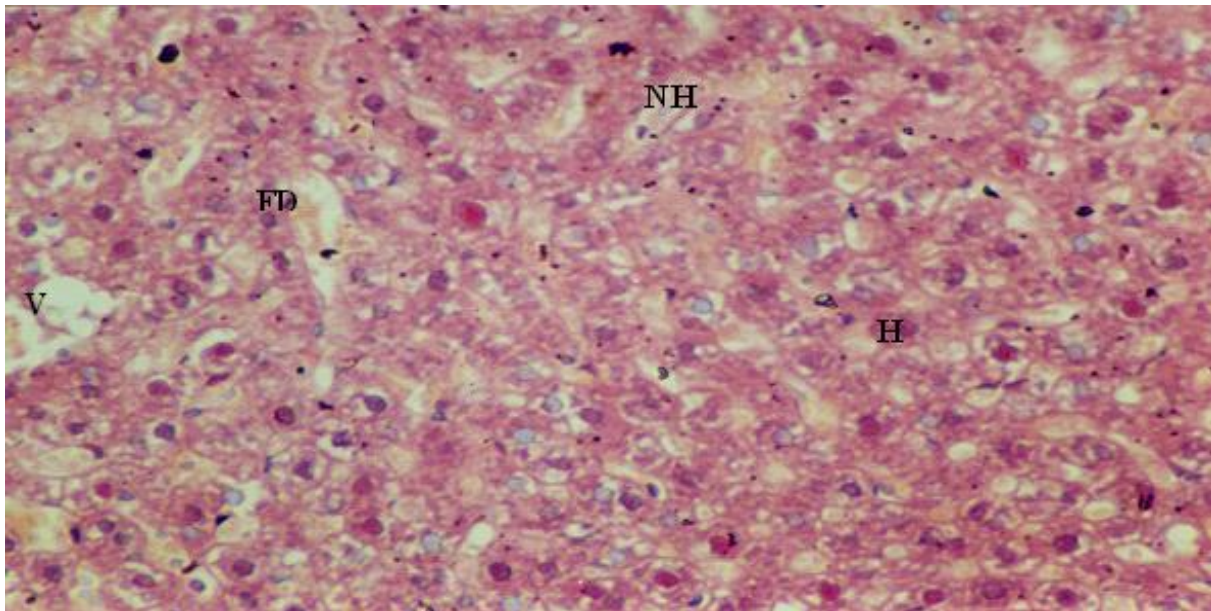
Figure 6. Photograph of the liver of alcoholic extract treated (*c. oxyacantha* seeds) rat at 40X showing normal hepatocyte (H), necrotizing hepatocytes (NH) vacuolization (V) and Fibrosis (hepatoprotective studies).
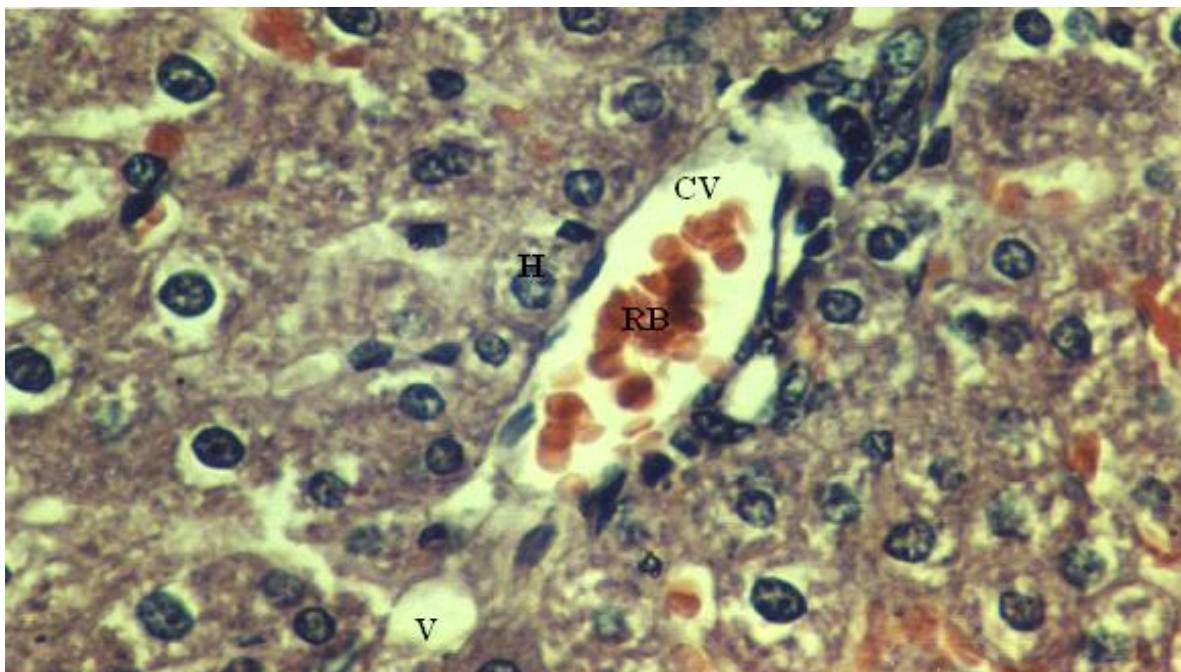

Figure 7. Photograph of the liver of alcoholic extract treated (*c. oxyacantha* seeds) rat at 40X showing normal hepatocyte (H), central vein (CV), vacuolization (V) and red blood cells (RB) (hepatocurative studies).

inhibitor of the microsomal drug metabolizing enzymes (MDME) (Shin, 1989 and Gopinathan *et al.*, 2004 and Hui-Mei *et al.,* 2008). As it is widely accepted that CCl_4 is metabolically activated by hepatic microsomal cyto-chrome P_{450} medicated reactions to the trichloromethyl radical (Slater, 1984). Therefore, the inhibitors of cytochrome P_{450} (MDME) can impair the bioactivation of CCl_4 into its toxic species and thus provide protection against hepatocellular damage (Nelson et al., 1980). Cytochrome P_{3A2} is one of the most abundantly expressed cytochrome, which metabolizes numerous drugs including barbiturates (Desjardins and Iversen,

1995).

The hepatocurative activity in alcoholic extract of *C. oxyacantha* seeds may be due to the presence of certain antioxidants which act as scavengers and remove the free radicals formed. These antioxidants also have the ability to prevent the process of peroxidation and improve the health of hepatocytes. Moreover, it is also observed that drugs that lower triglycerides such as fibrates improve hepatic biochemical parameters and tests (Parra, 2003; Hui-Mei et al., 2008).

The future prospective includes: 1) Characterization and quantification of pharmacologically active agents in the extracts and studies of their physiological effects such as; a) antibacterial activity; b) antifungal activity; c) anti-inflammatory activity; d) anticancerous activity; 2) formulation of drugs using active agents isolated from the extracts for trials.

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