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Assessment of Antihepatotoxic Effect of *Cuscuta californica* against Carbon Tetra Chloride Induced Liver Damage in Wistar Rats

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ABSTRACT

The plant *Cuscuta californica* was traditionally used to treat liver, spleen and gallbladder disorders such as jaundice and to support liver function in other Western herbalism. The present study was conducted to evaluate hepatoprotective effect of methanolic extracts of *Cuscuta californica* at doses of 100, 200 and 400 mg kg^{-1} day^{-1} and aqueous extract at dose 400 mg kg^{-1} day^{-1} orally for 5 days against carbon tetrachloride (CCl_4)- induced liver damage in rats. After injection of CCl_4 on 3rd day and administration of 100 mg kg^{-1} methanol extract for 5 days slightly increased the liver enzymes and decreased bilirubin concentration. At 200 and 400 mg kg^{-1} methanol extract and 400 mg kg^{-1} aqueous extract there was a damage and necrosis in the liver. The characteristic trait of extracts of plant is its toxicity on liver, kidney and changes on growth rate. This toxicity was accompanied by increase in the activity of aspartate transaminase (AST), alkaline phosphatase (ALP) and alanine transaminase (ALT), decreased concentration of albumin in serum and granulocytes and increased value of lymphocytes in blood. This study revealed that the plant *C. californica* did not showed any hepatoprotective activity but slight hepatotoxicity to the treated animals.

Key words: *Cuscuta californica,* hepatotoxicity, silibin, Kupffer cells, cholestasis, convolvulaceae

INTRODUCTION

The toxicity of plants to human, farm animals, birds and rodents is according to their chemical constituents, toxicity occurs when these plants were taken in large quantities and has been investigated in many countries (Adam *et al*., 2011b).

Hepatoprotection or antihepatotoxicity is the ability to prevent damage to the liver (Dehmlow *et al*., 1996). Many toxins target the liver and causes hepatotoxic effects that can be observed by biochemical parameters such as enzymes and proteins that synthesized by the liver.

Many herbs have been proven to be hepatoprotective and used in folk medicine but no scientific evidence to support such claims is available yet (Al-Qasoumi, 2007).

Synthetic and herbal drugs come mainly from medicinal herbs. In the commercial market, medicinal herbs are used as raw drugs, tinctures or extracts. Active constituents that are isolated from those herbs used for applied research (Malhotra and Singh, 2007).

C. californica, family: Convolvulaceae, Hamool in Arabic: Common name: California dodder, the genus is found throughout the temperate to tropical regions of the world, with the greatest species diversity in subtropical and tropical regions and in Sudan found in Nile bank and Gezira occasionally (Braun *et al*., 1991).

Phytochemical analysis of nine species of the genus *Cuscuta* yielded characteristic patterns of soluble phenolic constituents. These compounds can be used for classification of each species. The specific patterns proved to be stable in all *Cuscuta* ssp. Irrespective of host plant or localities (Loffler *et al*., 1997).

The active constituents in Dodder seed are thought to be a group of flavonoids. The seed contains quercetin and many others like cuscutin, astragalin, vitamin A, hyperosides and sterols (Draco Extracts Facts, 2002). Other researches done in Asian universities indicated that *Cuscuta* seeds contain a complex carbohydrate that stimulates the immune system and has some antioxidant properties as well (Bradley, 2007).

Despite the fact that *Cuscuta* is unpopular with farmers, it has a long history of folk use, in traditional Western claims for *Cuscuta* are that it is a mild laxative, a mild diuretic, sore knees, frequent urination, lower back pain, dry eyes blurred vision and that it can be used to treat scurvy and sciatica (Molony, 1998). The plant was also traditionally used to treat liver, spleen and gallbladder disorders such as jaundice; and to support liver function in other Western herbalism. It is still used, although rarely, in that way by modern herbalists (Molony, 1998).

The present study was therefore, planned to evaluate the hepatoprotective effect of methanolic and aqueous extracts of *C. californica* against carbon tetrachloride induced liver damage in rats orally. Emphasis was placed on comparative changes in growth, clinical abnormalities, lesions and alterations in haematological and serobiochemical constituents of treated rats.

MATERIALS AND METHODS

Plant material: *C. californica* was collected from Khartoum state, Sudan in March, 2011. The plant tissues were cleaned, shade-dried and ground with a mechanical grinder.

Preparation of plant extracts: Plant extraction was performed by using Soxhlet apparatus. The air-dried areal part of the plant was triturated to coarse powder, 50 g of the powder was weighed precisely and subjected to extraction with petroleum ether (60-80°C) for 2 h, then, the extract was separated from solvent using rotary evaporator, the plant residues were further dried, weighted and extracted with methanol (99.8%) for 2 h, then, the extract was separated from solvent using rotary evaporator, the plant residues were further dried, weighted and extracted with distilled water over night at room temperature (25-30°C), filtered and dried by freeze dryer (Culei, 1989).

Experimental design: Forty two, 4 weeks old male Wistar rats and of 86±10 g average body weight were used. The rats were clinically healthy and housed within the premises of the Faculty of Science and Technology, Al-Neelain University, Khartoum and fed on rat diet (flour 50.3%, meat 40%, edible oil 7.5%, sodium chloride 1.5% and vitamins and minerals 0.7) and water provided *ad libitum*.

Animals were divided into seven groups, each group containing six animals. group 1 represent control, received distilled water for 5 days. group 2 represent induction control, received carbon tetra chloride (CCl_4) 3 mL kg^{-1}, subcutaneously (s.c.), 1:1 dilution with olive oil on 3rd day.

Group 3 received silymarin (50 mg kg^{-1} day^{-1} orally) for 5 days and CCl_4 induction on 3rd day. Groups 4, 5 and 6 received (100, 200 and 400 mg kg^{-1} day^{-1} orally) methanol extract of the plant, respectively and group 7 received aqueous extract (400 mg kg^{-1} day^{-1}) for 5 days and CCl_4 induction on 3rd day (s.c.). Group 6 received 400 mg kg^{-1} aqueous extract for 5 days orally and CCl_4 induction on 3rd day (s.c.). Clinical signs, average body weight and body weight gain were reported for each group. On the 6th day, animals were killed under diethyl ether anaesthia and blood samples were immediately collected and divided into two parts, one for hematology analysis and the other part separated by centrifugation for serology analysis. At necropsy, all rats were examined to identify gross lesion. And the specimens of the liver, heart, spleen, kidney and small intestine were quickly removed after autopsy and fixed in 10% formalin for histopathological study.

Haematological methods: Whole blood for hematological parameters collected in EDTA anticoagulant blood container and examined for Haemoglobin (Hb), Packed Cell Volume (PCV), Red Blood Cells (RBCs), White Blood Cells (WBCs) and differential WBCs count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) and the measuring techniques were performed according to an Automated Hematology Analyzer (Human GambH, Max-Planck-Ring 21, D-65205. Wiesbaden, Germany).

Serobiochemical methods: Blood samples were collected and allowed to clot and sera were separated by centrifugation at 3000 rpm for 5 min and stored at -20°C until analysed. Serum samples were analyzed for the activities of the liver enzymes, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and for concentrations of total protein, albumin, globulin and bilirubin using Roche Diagnostic Hitachi 902 Analyser, Germany.

Pathological methods: Necropsy was made immediately after slaughter to identify gross lesions and specimens of heart, stomach, liver, kidneys, intestines and spleen were fixed in 10% neutral buffered formalin and processed for histopathology (dehydrated in gradual alcohols (30, 50, 70, 90 and 100%), embedded in paraffin wax for supporting and sections of 5 μ were prepared by microtome) and stained with hematoxylin and eosin (H and E) (Andrew *et al.,* 2008).

Statistical analysis: Statistical Package for Social Science (SPSS, version 16) was used for the analysis of the data. The significance of differences between means was compared among the groups using Independent-sample t-test (Snedecor and Cochran, 1989). Values were expressed as Mean±Standard error.

RESULTS

Growth changes: The effect on body weight and body weight gain of rats given daily oral doses of *C. californica* extracts (aqueous and methanolic) at 100, 200 and 400 mg kg^{-1} (groups 4-7) for 5 days are summarized in Table 1. The control rats (group 1) had the higher ($p<0.05$) body weight gains than methanol extracts (groups 4, 5 and 6) and aqueous extract (group 7) at 6th day after treatment, but no significant changes with group 3 were observed (Silymarin control).

Haematological changes: After 5 days of treatment, the values of Hb, MCH, MCHC, WBCs and granulocytes were higher ($p<0.05$), lymphocytes were lower ($p<0.05$) in group 2 than control (group 1), Hb and PCV values were lower ($p<0.05$) in group 6, WBCs and Granulocytes were

Table 1: Body weight and Body weight gain in rats orally given *C. californica* extracts for 5 days

	Body weight (g)	
Treatment groups	0 day	5 days
Control (normal diet)	93.0±1.2	10.2±1.4
CCl_4 control	92.5±3.4	8.2±1.3*
Silymarin control	85.8±2.7	9.3±2.7^{ns}
Methanolic extract (100 mg kg^{-1} day^{-1})	96.0±2.4	6.4±1.7*
Methanolic extract (200 mg kg^{-1} day^{-1})	92.5±1.1	6.1±1.2*
Methanolic extract (400 mg kg^{-1} day^{-1})	86.6±2.2	3.2±1.1*
Aqueous extract (400 mg kg^{-1} day^{-1})	91.8±2.3	6.8±2.2*

Values are Mean±SE, ns: Not significant, *Significant at $p<0.05$

higher ($p<0.05$), lymphocytes were lower ($p<0.05$) in group 5 than control (group 1). The values of Hb, MCH, MCHC, WBCs and Granulocytes in groups 4, 6 and 7 were lower ($p<0.05$). Hb, MCH and MCHC were lower ($p<0.05$) in group 5 and lymphocytes were higher ($p<0.05$) in groups 4, 6 and 7 than CCl_4 control (group 2). The values of PCV in group 7 and WBCs in group 6 were lower ($p<0.05$), WBCs in group 5 were higher ($p<0.05$) than silymarin control, lymphocytes values were higher ($p<0.05$) in group 6 and 7 and Granulocytes were lower ($p<0.05$) than silymarin control and other groups (Table 2).

Serobiochemical changes: After 5 days of treatment, the activity of AST and ALT was higher ($p<0.05$) in all groups, activity of ALP was higher ($p<0.05$) in groups 2, 5 and 6 than control (group 1) and lower ($p<0.05$) in group 3 and group 4, concentration of total protein was lower ($p<0.05$) in group 7, concentration of albumin was lower ($p<0.05$) in group 6 and 7, concentration of globulin was higher ($p<0.05$) in group 3 and group 6 and bilirubin was lower ($p<0.05$) in group 4 and group 7 than control (group 1). The activity of AST, ALP and concentration of bilirubin were lower ($p<0.05$) in group 4 than CCL_4 control (group 2). The activity of AST was higher ($p<0.05$), ALP was lower ($p<0.05$) in groups 5, 6 and 7, the activity of ALT was higher ($p<0.05$) in group 5 and group 7, concentration of total protein, albumin and bilirubin were lower ($p<0.05$) in group 7, concentration of globulin was higher ($p<0.05$), albumin was lower ($p<0.05$) in group 6. Bilirubin was lower ($p<0.05$) in group 4 than CCl_4 control. The activity of AST and ALP was higher ($p<0.05$) in groups 5, 6 and 7, the activity of ALT was lower ($p<0.05$) in group 4 and group 6, concentration of total protein and albumin were lower ($p<0.05$) in group 6 than Silymarin control (group 3) (Table 3).

Pathological changes: After 5 days of treatment of the daily oral doses of *C. californica* extracts. There were no lesions in the spleen, heart and other vital organs of control rats (group 1) and Silymarin control (group 3) and there were lesions in the liver, kidney and intestine of rats given CCl_4 control (group 2), there is fatty cytoplasmic vacuolation of the centirlobular hepatocytes and cell necrosis in the liver of 200 and 400 mg kg^{-1} methanolic extract (Fig. 1a, b) and fatty cytoplasmic vacuolation of the centirlobular hepatocytes, hemorrhage and necrosis in the liver of 400 mg kg^{-1} aqueous extract (Fig. 2) segmentation and packing of the glomerular tubules, dilatation and necrosis of the renal tubules of both 400 mg kg^{-1} methanol and aqueous extract (Fig. 3), infiltration of lymphocytes and congestion of intestinal blood vessels.

Table 2: Haematological analysis of rats given *C. californica* methanol and aqueous extract orally for 5 days

	Groups						
Parameters	Control (normal diet)	CCl_4 control	Silymarin control	*C. californica* (100 mg kg^{-1} day^{-1}) methanol extract	*C. californica* (200 mg kg^{-1} day^{-1}) methanol extract	*C. californica* (400 mg kg^{-1} day^{-1}) methanol extract	*C. californica* (400 mg kg^{-1} day^{-1}) aqueous extract
Hb (g dL^{-1})	16.9±0.5	20.7±0.9^{a}	16.3±1.4^{ns}	15.7±0.2^{b}	17.3±0.5^{b}	14.5±0.5^{ab}	15.7±1.2^{bc}
RBCs ($\times 10^{6}$ mm^{3})	10.4±0.5	10.1±0.4^{ns}	10.8±1.4^{ns}	10.0±0.1^{ns}	10.3±0.2^{ns}	9.1±0.5^{ns}	9.6±0.7^{ns}
PCV (%)	68.3±2.6	63.9±3.7^{ns}	69.9±9.6^{ns}	63.4±1.5^{ns}	64.6±1.6^{ns}	56.1±3.9^{ab}	60.9±2.3^{ac}
MCV (m^{3})	65.4±0.7	62.7±0.3^{ns}	64.2±0.2^{ns}	63.2±0.8^{ns}	62.3±0.4^{ns}	61.4±1.2^{ns}	63.0±0.7^{ns}
MCH (pg)	16.2±0.3	20.4±0.2^{a}	15.2±0.6^{ns}	15.6±0.1^{b}	16.9±0.3^{b}	15.9±0.1^{b}	16.1±0.4^{b}
MCHC (%)	24.8±0.3	32.6±0.3^{a}	23.7±1.0^{ns}	24.9±0.2^{b}	26.7±0.2^{b}	26.0±0.3^{b}	25.7±0.6^{b}
WBCs ($\times 10^{3}$ mm^{3})	10.0±1.6	17.9±2.0^{a}	11.3±1.8^{ns}	11.9±2.1^{b}	14.7±0.2^{ac}	9.4±0.9^{bc}	9.9±0.9^{b}
Lymphocytes (%)	53.9±0.7	39.7±0.5^{a}	49.1±6.0^{ns}	50.5±4.5^{b}	45.4±0.3^{a}	56.9±2.0^{bc}	58.8±3.4^{bc}
Granulocytes (%)	46.1±0.7	60.3±0.5^{a}	50.9±0.6^{a}	49.9±4.5^{b}	54.5±0.3^{a}	43.1±2.0^{bc}	41.1±0.3^{bc}

Values are Mean±SE, ns: Not significant, ^{a}Significant for normal control, ^{b}Significant for CCl_4 control, ^{c}Significant for silymarin control ($p<0.05$)

Table 3: Serobiochemical analysis of rats given *C. californica* methanol and aqueous extracts orally for 5 days

	Groups						
Parameters	Control (normal diet)	CCl_4 control	Silymarin control	*C. californica* (100 mg kg^{-1} day^{-1}) methanol extract	*C. californica* (200 mg kg^{-1} day^{-1}) methanol extract	*C. californica* (400 mg kg^{-1} day^{-1}) methanol extract	*C. californica* (400 mg kg^{-1} day^{-1}) aqueous extract
AST (IU)	9.5±0.7	26.8±0.2^{a}	20.7±0.4^{a}	18.3±0.5^{ac}	65.6±0.2^{abc}	39.1±0.2^{abc}	38.8±0.7^{abc}
ALT (IU)	6.4±1.4	11.6±1.3^{a}	20.8±1.4^{a}	13.5±1.4^{ab}	22.3±1.9^{a}	12.1±1.3^{abc}	18.4±1.7^{ac}
ALP (IU)	44.3±6.0	93.4±5.8^{a}	28.0±5.9^{a}	27.8±5.5^{ac}	81.0±5.9^{abc}	76.4±4.5^{ab}	44.4±5.5^{bc}
Total protein (g dL^{-1})	6.8±0.5	6.8±0.3^{ns}	8.2±0.4^{b}	7.3±0.3^{ns}	7.0±0.3^{ns}	7.2±0.8^{ns}	5.5±0.6^{c}
Albumin (g dL^{-1})	4.8±0.4	4.4±0.5^{ns}	4.6±0.9^{ns}	4.2±0.7^{ns}	4.3±0.3^{ns}	3.3±0.2^{abc}	3.4±0.3^{abc}
Globulin (g dL^{-1})	2.4±0.5	2.4±0.6^{ns}	3.5±0.1^{a}	3.1±0.6^{ns}	2.7±0.5^{ns}	3.8±0.4^{ac}	2.0±0.6^{b}
Bilirubin (mg dL^{1})	1.7±0.7	1.2±0.3^{ns}	1.8±0.3^{ns}	0.7±0.3^{abc}	1.1±0.2^{ns}	1.8±0.9^{ns}	0.7±0.2^{abc}

Values are Mean±SE, ns: Not significant, ^{a}Significant for normal control, ^{b}Significant for CCl_4 control, ^{c}Significant for silymarin control ($p<0.05$)

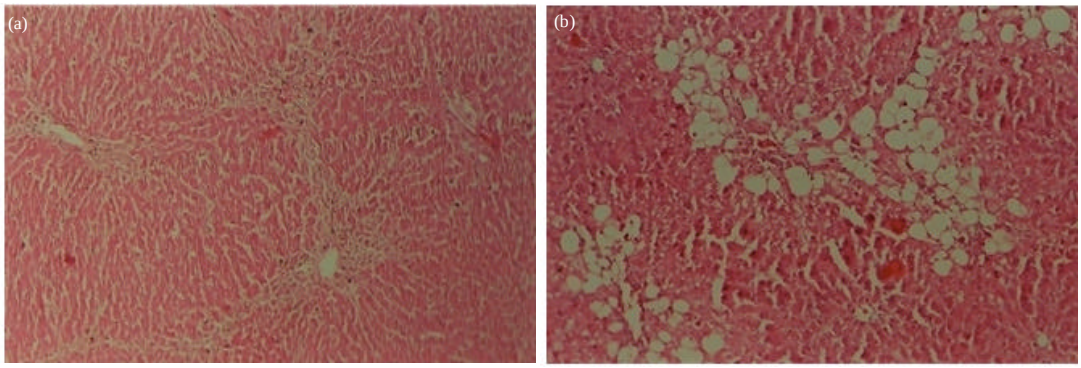

Fig. 1(a-b): Liver of rats receiving daily oral doses of *C. californica* methanol extract (400 mg kg^{-1}) for 5 days showing (a) Fatty change of the centirlobular hepatocytes and necrosis of the hepatocytes and (b) Fatty cytoplasmic vacuolation, H and E, X100

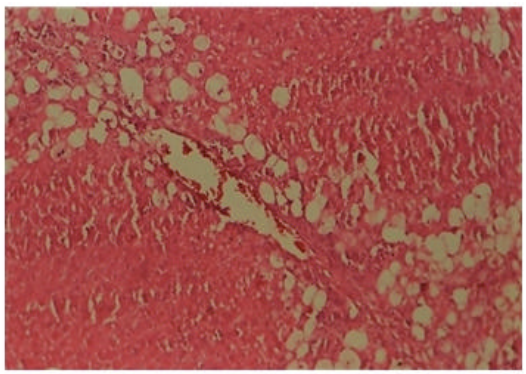

Fig. 2: Liver of rats receiving daily oral doses of *C. californica* aqueous extract (400 mg kg^{-1}) for 5 days showing fatty cytoplasmic vacuolation of the centirlobular hepatocytes, hemorrhage and necrosis, H and E, X100

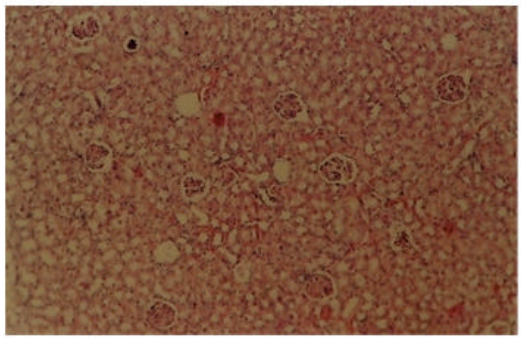

Fig. 3: Kidney of rats receiving daily oral doses of *C. californica*, methanol extract (400 mg kg^{-1}) for 5 days showing glomerular alteration, necrosis, segmentation and packing of glomerular tubules, H and E, X100

DISCUSSION

Changes associated with CCl_4-induced liver damage are similar to that of acute viral hepatitis (Suja *et al*., 2004), CCl_4-mediated hepatotoxicity was chosen as the experimental model. The ability of a hepatoprotective drug to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms that have been disturbed by a hepatotoxin is the index of its is protective effects (Yadav and Dixit, 2003). The hepatotoxicity induced by CCl_4 is due to its metabolite, a free radical which affects the cellular permeability of hepatocytes, hepatocellular necrosis leads to elevation of the serum marker enzymes, which are released from the liver into blood (Shenoy *et al*., 2001). Adam *et al*. (2011a) stated that methanol extract of *Argemone mexicana* revealed significant hepatoprotective activity through reducing the serum marker enzymes, like AST, ALT and ALP. Also histopathological studies confirmed the hepatoprotective activity of methanol extract. *Argemone mexicana* L. indeed has a reasonable potential in healing liver parenchyma and regeneration of liver cells hence it may acts as a potent liver tonic, these results is opposite to that obtained by the plant under study which according to results shows no hepatoprotective activity.

This study was designed to show hepatoprotective effect of methanolic and aqueous extracts of *C. californica* against carbon tetrachloride (CCl_4)-induced liver damage in experimental rats orally at a doses of 100, 200 and 400 mg kg^{-1} day^{-1}. The *Cuscuta* contains soluble phenolic constituents (Loffler *et al*., 1997). There is decrease of growth change, is due to mal absorption in intestinal desquamation or damage in other vital organs. Results of the present study showed that after injection of CCl_4 on 3rd day and administration of 100 mg kg^{-1} day^{-1} methanol extract of *C. californica* for 5 days slightly increased the liver marker enzymes and decreased bilirubin and at 200 and 400 mg kg^{-1} day^{-1} methanol extract and 400 mg kg^{-1} day^{-1} aqueous extract, there is a damage and necrosis in the liver attributed to the increased activity of ALT and ALP and the decreased concentration of albumin resulting from inability of hepatocytes to synthesize the enzyme and albumin insufficiency or over excretion in urine in renal dysfunction. An increase in activity of AST is attributed to the damage in liver and heart. There are changes in total protein and globulin due to loss of the liver functions.

This study revealed that the plant did not showed any hepatoprotective activity but slight hepatotoxicity for male Wistar rats from *C. californica* at concentration 100 mg kg^{-1} methanol extract and high hepatotoxicity at 200 and 400 mg kg^{-1} methanol extract and 400 mg kg^{-1} aqueous extract administered by oral route. Consumption of *C. californica* causes damage of vital organs exemplified by necrosis, fatty changes and hemorrhage.

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