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The hepato-ameliorating effect of *Solanum nigrum* against CCl_4 induced liver toxicity in Albino rats

Abd-Elraheim Ali Elshater, Muhammad Mahmud Ali Salman and Samar Ali Mohamed
Department of Zoology, Faculty of Science, South Valley University, Qena

ABSTRACT

The present study was investigated to evaluate the hepato-ameliorating and antioxidant activity of two aqueous extracts of *Solanum nigrum* (SN) against CCl_4-induced toxicity in rats. Male Albino rats were divided in four groups with 8 animals in each group. Group (1) was normal group and group (2) was injected intraperitoneal (i.p.) with CCl_4 (1ml/kg) 3 times weekly for 2 weeks (control). Group (3) was injected with CCl_4 and then treated with extract from whole plant of *Solanum nigrum* (500 mg/kg) and group (4) was injected with CCl_4 and then treated with extract from fruits of *Solanum nigrum* (250 mg/kg). CCl_4 injection enhanced activity of hepatic enzymes (AST and ALT) while it decreased serum total protein and albumin in experimental animals. It also decreased RBC, platelets count, PCV and Hb levels. However it increased WBC count. CCl_4 injection increased level of lipid peroxidation resulting in a decrease in the level of enzymatic and non enzymatic antioxidants. Treatment with two extracts of *Solanum nigrum* altered these changes to near normal levels. But hepato-ameliorating and antioxidant effects of extract of *Solanum nigrum* fruits were found to be better than those of extract from whole plant of *Solanum nigrum*.

Keywords: Carbon tetrachloride, *Solanum nigrum*, liver toxicity, Albino rats, fruits of *Solanum nigrum*, antioxidant enzymes.

INTRODUCTION

Various studies have demonstrated that carbon tetrachloride (CCl_4) intoxication causes free radical generation in many tissues such as liver, kidney, heart, lung, testis, brain and blood (Rechnagel *et al*., 1989; Kumar *et al*., 2005; Khan and Ahmed, 2009 and Khan *et al*., 2009). CCl_4 has been commonly used as a hepatotoxin in experimental hepatopathy (Hsu *et al*., 2008 and Geetha *et al*., 2008) because it induced a cirrhotic response in animals which is similar to human cirrhosis of the liver (Taira *et al*., 2004; Lee *et al*., 2007 and Rudnicki *et al*., 2007). CCl_4-induced hepatic injury has been extensively used in animal models to evaluate the therapeutic potential of drugs and dietary antioxidants (Hsu *et al*., 2010). From thousands of years, herbal medicines have been widely used as hepatoprotective and anti-fibrotic drugs in the treatment of liver diseases (Dhiman and Chawla, 2005; Lee *et al*., 2007a and b and Lin *et al*., 2011). *Solanum nigrum* L. (SNL), belonging to the nightshade of the Solanaceae family (Ji *et al*., 2008). *Solanum nigrum* contains steroidal glycosides, steroidal alkaloids, steroidal oligoglycosides, solamargine and solasonine (Saijo *et al*., 1982). *Solanum nigrum* fruit extracts are reported to have hepatoprotective activity against CCl_4-induced hepatic damage (Raju *et al*., 2003). *Solanum nigrum* exerts protection against thioacetamide-induced liver fibrosis in mice (Hsieh *et al*., 2008). *Solanum nigrum* leaves are a potential source of antioxidants and help in reducing reactive oxygen species (ROS) levels (Radha *et al*., 2009). Also *Solanum nigrum* extract increased Hb and PCV levels and RBCs count and increased platelets, WBCs count (Vigila and baskaran, 2011).

MATERIALS AND METHODS

Materials

Chemicals

CCl_4 is a colorless non-flammable liquid, of molecular weight 153.84 was obtained from El-Nasr Pharmaceutical Chemical Co., A. R. E. Laboratory chemical division.

Plant material (*Solanum nigrum*):

A-Extract from the whole plant:

The whole plant of *Solanum nigrum* was collected from south valley university, Qena. Plants were powdered and prepared according to (**Lin *et al.*, 2008**).

Preparation of extract from fruits of *Solanum nigrum* (SN):

Ripe fruits were dried and finely powdered. Fruit extract was prepared according to (**Arulmozhi *et al.*, 2011**).

Animals:

32 adult male Albino rats weighing about (260-300g) were divided into four groups (8 rats/cage) in room temperature, for four weeks before starting the experiment, under natural day and night periods and supplied with a balanced stable commercial diet and water.

Methods

Experimental design:

The experimental animals were divided into 4 groups, 8 rats for each group.

Group 1: The rats were received orally NaCl 0.9% (normal group).

Group 2: The rats were injected intraperitoneal (i.p.) with carbon tetrachloride (CCl_4) (1 ml/kg), 3 times weekly, for 2 weeks (control group).

Group 3: (CCl_4+ *Solanum nigrum* extract): The rats were injected intraperitoneal (i.p.) with CCl_4 (1 ml/kg body weight), 3 times weekly for 2 weeks, following with oral administration of *Solanum nigrum* extract (500 mg/kg body weight) daily for 30 days.

Group 4: (CCl_4+extract from *Solanum nigrum* fruits): The rats were injected intraperitoneal (i.p.) with CCl_4 (1 ml/kg body weight), 3 times weekly for 2 weeks, following with oral administration of extract of *Solanum nigrum* fruits (250 mg/kg body weight) daily for 30 days.

At the end of experiment, all animals were sacrificed and the blood from every animal was taken into clean tubes. The blood, serum and liver tissue were collected from animals for hematological and biochemical analysis, respectively. Liver was removed, cleared off blood and immediately transferred to ice-cold containers containing 0.9% NaCl. Tissues were homogenized in 5ml of the phosphate buffer (K_2HPO_4, $K_2H_2PO_4$, EDTA and PVP) and centrifuged at 4000 rpm for 15min at 4 $^{\circ}$C. Then removed the supernatant which used for the estimation of various biochemical parameters.

Hematological studies: This blood was used for the examination of complete blood picture (platelets count, red blood cells count (RBCs), leukocytes count (WBCs), total hemoglobin and hematocrit assays) which done by Automated Hematology Analyzer (Diff3) Mek-6410/Mek-6420.

Assessment of biochemical parameters and antioxidants:

Alanine aminotranferease (ALT) and aspartate aminotransferase (AST) were determined according to Reitman and Frankel (1957), while albumin was determined according to Doumas *et al.*(1971), but total protein according to Gornal *et al*. (1949). Reduced glutathione (GSH) was determined according to Beutler *et al.* (1963), while super oxide-dismutase (SOD) was determined according Nishikimi *et al.* (1972), but Catalase (CAT) was determined according with Aebi (1984) and Malondialdehyde (MDA) was determined according to Ohkawa *et al.* (1979). All mentioned kits were bought from bio-diagnostic co. Giza, Egypt.

Statistical analysis:

All quantitative measurements were expressed as means ± SD of control and experimental animals. The data were analyzed using one way analysis of variance (ANOVA) on SPSS (statistical package for social sciences). Statistical significance was set up $P < (0.05)$.

DKRESULTS

Hematological results

As shown in Table (1) Hb content, PCV value and platelets count were highly significant decreased ($p \leq 0.01$) and RBCs count was significantly decreased ($p \leq 0.05$) while WBCs count was highly significantly increased ($P \leq 0.01$) in control group (CCl_4) as compared with normal group. Hb content and PCV value were significantly increased, however RBCs, platelets count increased in rats treated with both of whole plant extract of *Solanum nigrum* (G3) and extract of *Solanum nigrum* fruits (G4), while WBCs count was significantly decreased in (G3) and it decreased in (G4) when compared with control group.

Table 1: Effect of daily oral administration of the extracts of *Solanum nigrum* (500 mg/kg body weight) and fruits from *Solanum nigrum* (250 mg/kg body weight) after 30 days of treatment on complete blood picture (RBCs, WBCs, platelets, PCV% value and Hb content) of Albino rats, injected with CCl_4 (1 ml/kg b. w.) 3 times weekly for two weeks.

Groups	PCV (%)	Hb Conc. (g/dl)	RBCs ($x10^6/mm^3$)	WBCs ($x10^3/mm^3$)	Platelets ($x10^3/mm^3$)
	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D
Normal (G1)	48.12 ± 0.64	14.28 ± 0.63	6.02 ± 0.58	7.90 ± 1.07	695 ± 31.62
Control (CCl_4) (G2)	--a 36.70 ± 1.15	--a 10.38 ± 1.22	-a 4.91± 0.62	++a 19.27 ± 4.16	--a 263.50 ± 44.47
CCl_4 + *Solanum nigrum* extract (G3)	+b 42.25 ± 2.76	+b 12.72 ± 1.04	5.33± 1.67	-b 13.57 ± 2.75	264.125 ±38.86
CCl_4 + extract of *Solanum nigrum* fruits (G4)	+b 46.62 ± 2.5	+b 12.71 ± 1.48	5.88 ± 0.39	15.85 ± 2.64	341.50 ± 2.64

Results are expressed as mean ± S.D. of 8 animals.

+a = significantly increased compared with the normal $P < 0.05$ ++a = highly significant increased compared with the normal $P < 0.01$

-a = significantly decreased compared with normal $P< 0.05$ --a = highly significant decreased compared with normal $P < 0.01$

+b = significantly increased compared with control $P < 0.05$ ++b = highly significant increased compared with control $P < 0.01$

-b = significantly decreased compared with control $P < 0.05$ --b = highly significant decreased compared with control $p < 0.01$

Biochemical results:

The activity of serum ALT and AST were highly significant increased while serum albumin was significantly decreased and serum total protein was highly significant decreased in control group (CCl_4) as compared with normal group as shown in Table (2). A highly significant decrease in ALT and a significant decrease in AST showed in group treated with CCl_4 and *Solanum nigrum* extract (G3), while there was a highly significant decrease in serum AST and a significant decrease in ALT level in group treated with CCl_4 and extract of *Solanum nigrum* fruits (G4) as compared with control group. Serum albumin was significantly increased in (G3) and (G4) while serum total protein was highly significant increased in (G4), however it increased in (G3) when compared with control group.

Table 2: Effect of oral administration of daily doses of extracts of *Solanum nigrum* (500 mg/ kg body weight) and fruits from *Solanum nigrum* (250 mg/kg body weight) after 30 days of treatment on serum levels of ALT, AST, albumin and protein in Albino rats, injected with CCl_4 (1 ml/kg b. w.) 3 times weekly for two weeks.

Groups	ALT (Units/ml)	AST (Units/ml)	Albumin (g/dl)	Protein (g/dl)
	Mean ± S.D	Mean± S.D	Mean ± S.D	Mean ± S.D
Normal (G1)	15.25 ± 2.05	17.00 ± 1.92	3.95 ± 0.09	9.94 ± 1.07
Control (CCl_4) (G2)	++a 56.50 ± 3.07	++a 66.25 ± 5.20	-a 2.60 ± 0.26	--a 5.91 ±0.29
CCl_4 + *Solanum nigrum* extract (G3)	--b 23.37 ± 2.87	-b 35.00 ± 4.33	+b 3.77 ± 0.86	6.70 ± 0.60
CCl_4 + Extract of *Solanum nigrum* fruits (G4)	-b 23.62 ± 2.32	--b 26.93 ± 2.88	+b 3.70 ± 0.29	++b 8.76 ± 0.21

Results are expressed as mean ± S.D. of 8 animals.
+a = significantly increased compared with the normal P <0.05 ++a = highly significantly increased compared with the normal P< 0.01
-a = significantly decreased compared with normal P < 0.05--a = highly significant decreased compared with normal P < 0.01
+b = significantly increased compared with control P < 0.05 ++b = highly significant increased compared with control p < 0.01
- b = significantly decreased compared with control P <0.05 --b = highly significant decreased compared with control p < 0.01

The results recorded in Table (3) revealed that the activities of liver GSH, catalase and SOD were highly significant decreased while level of liver MDA was highly significant increased in control group (CCl_4) as compared with normal group. The activities of liver GSH and catalase were significantly increased but SOD activity was highly significant increased in group (G3) treated with CCl_4 and *Solanum nigrum* extract, but there was a highly significant increase in activities of liver GSH and catalase and a significant increase in SOD activity in group (G4) treated with CCl_4 and extract of *Solanum nigrum* fruits when compared with control group. The level of liver MDA was significantly decreased in group (G3) but it was highly significant decreased in group (G4) as compared with control group.

Table 3: Effect of daily oral administration of the extracts of *Solanum nigrum* (500 mg/kg body weight) and fruits from *Solanum nigrum* (250 mg/kg body weight) after 30 days of treatment on GSH, SOD and catalase activities and level of MAD in liver tissue of Albino rats, injected with CCl_4 (1 ml/kg b. w.) 3 times weekly for two weeks.

Groups	SOD (u/g)	Catalase (u/g)	MAD (nmol/l)	Glutathione (mg/g)
	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D
Normal animals (G1)	451.046 ± 7.94	1.977 ± .007	0.625 ± 0.166	8.305 ± 0.529
Control (CCl_4) (G2)	--a 341.50 ± 10.32	--a 0.220 ± 0.038	++a 24.943±2.235	--a 0.587±0.135
CCl_4 + *Solanum nigrum* extract (G3)	++b 449.87± 1.64	+b 1.942± 0.033	-b 4.518±1.722	+b 6.055± 0.456
CCl_4 + Extract of *Solanum nigrum* fruits (G4)	+b 440.25 ± 18.35	++b 1.966 ± 0.007	--b 0.680 ± 1.162	++b 7.282± 0.594

Results are expressed as mean S.D. of 8 animals.
+a = significantly increased compared with the normal p <0.05 ++a = highly significantly increased compared with the normal p< 0.01
-a = significantly decreased compared with normal p < 0.05 --a = highly significant decreased compared with normal p < 0.01
+b = significantly increased compared with control p < 0.05 ++b = highly significant increased compared with control p < 0.01
-b = significantly decreased compared with control p < 0.05 -b = highly significant decreased compared with control p< 0.01

The discussion

The toxicity of CCl_4 results from its reductive dehalogenation by the cytochrome p450 enzyme system into trichloromethyl free radical, which readily interacts with molecular oxygen to form the trichloromethyl peroxy radical (Williams and Burk, 1990). Both radicals are capable of binding to proteins or lipids leading to membrane lipid peroxidation and finally cell necrosis (Brattin *et al*., 1985 and Rechnagel *et al*., 1989). In the present study, carbon tetrachloride (1 ml/kg body weight) decreased PCV, Hb levels, platelets count and RBCs count. This depression in RBCs count and Hb content could be attributed to disturbed hematopoiesis, destruction of erythrocytes, reduction in the rate of their formation and /or their enhanced removal circulation (Essawa *et al*., 2010). Injection of CCl_4 increased WBCs count. This may be attributed to the defensive mechanism of immune system (Patrick-Iwuanyanw *et al*., 2007) so the ability of free radicals to increase WBCs count indicates that these radicals to an extent affected the defense mechanism of treated rats (Oluyemi *et al*., 2007). Administration of both the two extracts of *Solanum nigrum* (whole plant extract and fruits extract) altered these changes. It may be due to the presence of active constituents present in *Solanum nigrum* which stimulates the maturation and development of RBCs which in turn increases the level of Hb and PCV (Vigila and Baskaran, 2011).There is high content of ascorbic acid in *Solanum nigrum* (Mahanom *et al.,* 1999) which plays an important role in iron absorption and its transport. So it supplies iron for development and maturation of RBC. Also these constituents increased the platelets level. Injection of CCl_4 (1ml /kg. body weight) increased serum ALT and AST activities but it decreased serum albumin and total protein as compared with normal values. CCl_4 treated rats showed increase in activities of these enzymes, reflecting the damage of the liver cells or changes in the cell membrane permeability leading to leakage of enzymes from cells to the circulation (Botsoglou *et al*., 2008). While the diminution of total protein and albumin is due to liver damage induced by CCl_4 (Navarro and Senior, 2006). Administration of *Solanum nigrum* extract significantly decreased elevated enzymes and increased serum albumin and total protein. The reduction in the levels of these parameters toward the normal values by *Solanum nigrum* extract (SNE) is an indication of the stabilization of plasma membranes as well as repair of hepatic tissue damage caused by CCl_4 (Lin *et al*., 2008). This indicates the anti-lipid peroxidation of *Solanum nigrum* extract (SNE) which acted against the damaging effects of free radicals produced by CCl_4. Vigila and Baskaran (2011) observed that administration of extract of *Solanum nigrum* elevated albumin and total protein and it may be due to the presence of active constituents such as flavonoids and alkaloids which may prevent the excessive break down of protein. In present study, injection of CCl_4 produced oxidative stress as evidenced by a significant decrease in hepatic glutathione reduced (GSH) and super oxide dismutase (SOD) and catalase activities and increase of lipid peroxidation. Also in present study, CCl_4 increased MDA level. Treatment with *Solanum nigrum* increased hepatic SOD, catalase activities and GSH level while it decreased MDA level. High content of polyphenols, alkaloids and saponins in *Solanum nigrum* extract (SNE) (Muriel *et al*., 1992) contributes free radical scavenging and antioxidant activities. It has been demonstrated that water extract of *Solanum nigrum* Lin (SNL) contains several antioxidants, such as gallic acid,

catachin, caffeic acid, epicatechin, rutin and narigenin and possesses strong antioxidant activity in vitro (Lin *et al* 2008).

In conclusion, both of whole plant extract and fruit extract of *Solanum nigrum* exhibited a potent hepato-ameliorating and antioxidant effects in CCl_4-induced hepatotoxic rats. But hepato-ameliorating and antioxidant effects of extract of *Solanum nigrum* fruits were found to be better than those of extract from whole plant of *Solanum nigrum*.

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ARABIC SUMMARY

تأثير نبات عنب الديب ضد سمية الكبد المستحدثة بواسطة رابع كلوريد الكربون للتحسن الكبدي في الفئران البيضاء

عبد الرحيم على الشاطر ، محمد محمود على سالمان ، سمر على محمد حجاجي
قسم علم الحيوان- كلية العلوم – جامعة جنوب الوادي

لقد أوضحت الدراسة الحالية التأثير المُحسّن للمستخلصات المائية لنبات عنب الديب على سُميَّة الكبد المستحدثة بواسطة رابع كلوريد الكربون لذا استخدمت هذه الدراسة 32 فأر قُسّمت إلى 4 مجموعات كل مجموعة تضم 8 فئران ، عُدت المجموعة الأولى كمجموعة سيطرة أمَّا المجموعة الثانية تم حقنها برابع كلوريد الكربون (1مللى /كيلوجرام) 3 مرات أسبوعياً لمدة أسبوعين . أمَّا المجموعة الثالثة حقنت برابع كلوريد الكربون ومستخلص نبات عنب الديب (مستخلص النبات كله) أما عن المجموعة الرابعة فقد حُقنت برابع كلوريد الكربون ومستخلص ثمار نبات عنب الديب.

ونتيجة لحقن رابع كلوريد الكربون ارتفع نشاط إنزيمات الكبد وقل مستوى الالبيومين والبروتين في السيرم وقلَّ أيضا عدد كرات الدم الحمراء وعدد كرات الدم البيضاء و الصفائح ومستوى الهيموجلوبين وحجم الخلايا المتجمعة . كما أن الحقن برابع كلوريد الكربون زاد من عملية الأكسدة وقلل من مضادات الأكسدة في أنسجة الكبد ألا أنَّ التعامل بمستخلصات عنب الديب حسَّن هذه التغيرات الحادثة بواسطة رابع كلوريد الكربون ولكن تأثير مستخلص ثمار نبات عنب الديب خصيصاً أفضل من مستخلص النبات كله.