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Hepatoprotective Activity of Desert Truffle (*Terfezia claveryi*) in Comparison with the Effect of *Nigella sativa* in the Rat

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Abstract: Hepatoprotective activity of *Terfezia claveryi* aqueous, methanolic and petroleum ether extracts was evaluated in the rat using a potent hepatotoxin carbon tetrachloride (CCl_4) in comparison with the hepatoprotective activity of a reference plant *Nigella sativa*. The extracts were administrated *via gavage* three days prior to CCl_4 intoxication followed by two additional doses one hour and four hours after CCl_4 injection. Twenty four hours after intoxication, blood samples were collected and serum bilirubin concentration, Alkaline Phosphatase (ALP), Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) activities were measured. Body weight was measured then livers were excises and livers were weighed. The aqueous, methanolic and petroleum ether extracts of *T. claveryi* and *N. sativa* lowered all liver function tests significantly. However, the aqueous extract of *T. claveryi* almost normalized the effect of CCl_4 and was as effective as the petroleum ether extract of the reference plant *N. sativa*. Moreover, the aqueous extract of *T. claveryi* normalized CCl_4 induced hepatomegaly, which was comparable to the effect of petroleum ether extract of *N. sativa*. These results demonstrate that aqueous extract of *T. claveryi* possesses a very powerful hepatoprotective activity against CCl_4 and it is as effective as petroleum ether extract of the reference plant *N. sativa*.

Key words: Truffles, *Terfezia claveryi*, *Nigella sativa*, hepatoprotective, bilirubin, ALP, AST, ALT

INTRODUCTION

Truffles grow naturally in many parts of the world including particular localities of the Arabian Desert (Al-Delaimy, 1977). Truffles are considered one of the oldest foods used by the Arabs. They are well known for their nutritional importance especially when compared with meat and fish (Bokhary and Parvez, 1993). The Bedouins use truffles as a substitute for meat in their diet. Its preparation and cooking methods are similar to those of meat (Al-Delaimy and Abu-Ghraib, 1970). Truffles are healthy foods that are low in calories and fat and rich in fiber, proteins, vitamins and minerals. Their protein content is higher than that of most vegetables and their amino acid composition is comparable to that of animal proteins (Gazzani *et al*., 1998a; Gazzani *et al*., 1998b; Murcia *et al*., 2002).

Truffles are traditionally used in folk medicine for the treatment of eye ailments in Iraq, Saudi Arabia and the Eastern Badia of Jordan (Janakat *et al*., 2004). Furthermore, truffles have been used as convalescent for several centuries due to their high content of antioxidants such as vitamin A, C, ß-carotene and many phenolic compounds, which are very specialized scavengers of peroxy radicals and are able to reduce and chelate ferric ions, which induce lipid peroxidation (Gazzani *et al*., 1998a; Gazzani *et al*., 1998b; Murcia *et al*., 2002). The effect of truffles in general and of *T. claveryi* in particular on liver functions was not documented earlier. Since the overall incidence of liver diseases in the general population is about 1% (Rochling, 2001) and since truffles are very rich source of antioxidant then most probably truffles will act as a hepatoprotective agent. Therefore the present study was undertaken to evaluate the hepatoprotective activity of aqueous, methanolic and petroleum ether extracts of *T. claveryi* in comparison with a reference plant *N. sativa* extracts against experimental liver damage inflicted by CCl_4.

MATERIALS AND METHODS

Sample preparation: *Terfezia claveryi* which is dark brown red in color, small in size and round in shape was purchased from local markets of Baghdad. The sample was washed carefully, peeled and preserved at -20°C until use. *Nigella sativa* seeds were purchased from the local market of Irbid. The sample was sorted from impurities, washed and air-dried then was kept at room temperature until use.

Chemicals: Bilirubin, ALP, ALT and AST kits were purchased from Cromatest, Spain. CCl_4 was purchased from Pharmacos LTD, England.

Test animals: Male Wister albino rats weighing 170-200 g were obtained from the Animal House Unit at Jordan University of Science and Technology. The animals were

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housed in suspended screen wire cages in an air-conditioned room at 20±3°C and maintained on tap water and standard diet *ad libitum*. All animal experiments conformed to local animal care regulations.

Preparation of extracts: Frozen Iraqi truffles were homogenized using 1:3 (w/v) of each solvent (distilled water, methanol or petroleum ether), using a household blender on full speed for one minute. Whereas, *N. sativa* seeds were first milled using a household electric mill then the sample was mixed with each solvent using a household blender on full speed. The homogenates were refrigerated overnight, filtered through cheesecloth and then were centrifuged at 4000 rpm for 15 min. The supernatants were then dried using rotary evaporator. The dried matter of the aqueous and methanolic extracts were re-suspended using distilled water while the dried matter of the petroleum ether extracts were re-suspended using paraffin oil and kept at -20°C until use (Nielsen *et al*., 1997; Janakat *et al*., 2004).

Experimental design: Hepatotoxicity was induced in rats using a (1:1) mixture of CCl_4:olive oil, administrated intraperitoneally at a single dose of 2 ml CCl_4/kg body weight (Janakat and Al-Merie, 2002a,b). Rats were divided into groups of five. The control group consisted of normal untreated rats (negative control). The other four groups were intoxicated with CCl_4 as described above. Intoxicated groups were treated either with *T. claveryi* or with *N. sativa* extracts (aqueous, methanolic, or petroleum ether). One intoxicated group did not receive any extracts (positive control). The test groups were treated twice daily with the extracts using intragastric tube for three days. On the fourth day, the rats were intoxicated with CCl_4:Olive oil mixture intraperitoneally, followed by two additional doses of truffle extracts after 1 and 4 h of CCl_4 injection. The negative and positive control groups received distilled water instead of the extracts. Blood samples were collected 24 h after CCl_4 administration (Janakat and Al-Merie, 2002a,b).

Assessment of liver function: Rats were anaesthetized with ether and then decapitated for blood collection. Serum was separated by centrifugation at 3000 rpm for 10 min. The level of total serum bilirubin and the activity of ALP, ALT and AST were assayed according to the methods of Jendrassik and Groff (1938), Bergmeyer and Brent (1974), Reitman and Frankel (1957) and Berger and Rudolf (1963), respectively (Jendrassik and Groff, 1938; Bergmeyer and Brent, 1974; Reitman and Frankel, 1957; Berger and Rudolf, 1963).

Statistical analysis: Data were analyzed using analysis of variance of the complete randomized design (ANOVA) using the General Linear Model (GLM) of the Statistical Analysis System (SAS, 2004). Least significant difference was calculated by Students t-test. Different superscripts differ significantly $p<0.05$.

RESULTS AND DISCUSSION

Effect of *T. claveryi* extracts on liver function tests: Table 1 depicts the effect of *T. claveryi* extracts on liver function tests. As expected the positive control group which was intoxicated with the potent hepatotoxin CCl_4 had significantly higher bilirubin concentration (0.50 mg/dl) in comparison to the negative control group (0.14 mg/dl). This comes in accordance with the all researchers findings since the classical article of Recknagel, 1967 to the present day (Recknagel, 1967; Muchizuki *et al*., 2009). The elevation of these parameters is attributed to significant free radical mediated hepatotoxicity leading to cell necrosis, fibrosis and cirrhosis. The mechanism by which CCl_4 causes damage involves the biotransformation of CCl_4 by cytochrome P450 system into a trichloromethyl free radical (CCl_3), which in turn is transformed into a more reactive trichloromethyl peroxyl radical (CCl_3O_2) leading to lipid peroxidation and hepatocellular injury [18]. Moreover, ingestion of *T. claveryi* extracts caused a strong significant reduction in all liver function tests performed. Serum bilirubin level decreased from 0.5 to 0.16, 0.31 and 0.4 mg/dl in aqueous, methanolic and petroleum ether extracts respectively. Whereas, the activity of ALP decreased from 144-70, 105 and 126 U/L respectively, ALT decreased from 791-111, 356 and 511 U/L respectively and AST decreased from 795-188, 420, and 612 U/L respectively. This can be attributed to the high antioxidants contents in *T. claveryi*, such as vitamin C and ß-carotene (Gazzani *et al*., 1998a; Gazzani *et al*., 1998b; Murcia *et al*., 2002) which stop the mounting of peroxyl radical formation and preventing plasma membrane bleb formation, which conserve the integrity of the plasma membrane from rupturing and cytosolic enzymes such as ALP, ALT and ASP from being released into the blood stream (Mehendale *et al*., 1994).

Effect of *N. sativa* extracts on liver function tests: Table 2 depicts the effect of *N. sativa* aqueous, methanolic and petroleum ether extracts on liver function tests. Elevated bilirubin level induced by CCl_4 decreased significantly when aqueous, methanolic and petroleum ether extracts of *N. sativa* were used (from 0.49-0.34, 0.42 and 0.21 mg/dl respectively). The activity of ALP decreased from 142-105, 133 and 81 U/L respectively, ALT decreased from 781-385, 553 and 196 U/L respectively and the activity of AST decreased from 790-404, 601 and 210 U/L respectively. As evident form the above mentioned results all extracts were hepatoprotective, yet the hydrophobic extract was the most potent, this can be attributed to the volatile oil which is abundant in *N. sativa* seeds that has been

Table 1: Effect of *T. claveryi* extracts on liver function tests

Group	-ve control	+ve control	Aqueous extract	Methanolic extract	Petroleum ether extract
BRN (mg/dl)	0.14±0.003[e]	0.50±0.009[a]	0.16±0.005[d]	0.31±0.011[c]	0.40±0.007[b]
ALP (U/L)	46±1.034[e]	144±1.035[a]	70±1.409[d]	105±1.611[c]	126±1.034[b]
ALT (U/L)	108±1.234[d]	791±2.566[a]	111±1.235[d]	356±2.45[c]	511±1.232[b]
AST (U/L)	170±0.889[e]	795±2.18[a]	188±3.905[d]	420±1.235[c]	612±1.235[b]

ALP; Alkaline Phosphatase, ALT; Alanine Aminotransferase, AST; Aspartate Aminotransferase, BRN; Bilirubin,-ve control; Normal rats, +ve control; CCl_4 intoxicated rats. Values are expressed as mean±SEM (n = 5). P-values were calculated by Students t-test.
Means with superscripts (b,c,d,e) differ significantly from the positive control group, p<0.05

Table 2: Effect of *N. sativa* extracts on liver function tests

Group	-ve control	+ve control	Aqueous extract	Methanolic extract	Petroleum ether extract
BRN (mg/dl)	0.12±0.005[e]	0.49±0.009[a]	0.34±0.041[c]	0.42±0.008[b]	0.21±0.009[d]
ALP (U/L)	46±1.409[e]	142±1.784[a]	105±2.523[c]	133±1.596[b]	81±1.684[d]
ALT (U/L)	111±1.502[e]	781±1.491[a]	385±1.491[c]	553±2.016[b]	196±1.491[d]
AST (U/L)	171±1.235[e]	790±1.491[a]	404±1.127[c]	601±1.008[b]	210±1.127[d]

ALP; Alkaline Phosphatase, ALT; Alanine Aminotransferase, AST; Aspartate Aminotransferase, BRN; Bilirubin,-ve control; Normal rats, +ve control; CCl_4 intoxicated rats. Values are expressed as mean±SEM (n = 5). P-values were calculated by Students t-test.
Means with superscripts (b,c,d,e) differ significantly from the positive control group, p<0.05

shown to contain many antioxidants such as thymoquinone, monoterpenes (El-Tahir *et al*., 1993). *N. sativa* seeds extracts were also found to cause immunomodulatoion (El-Kadi and Kandil, 1987), act as anti-inflammatory agent (Houghton *et al*., 1995) anti-tumor agent (El-Daly, 1998) and prevents liver fibrosis, cirrhosis and decreases liver enzymes elevation induced by the potent hepatotoxin CCl_4 in the rat (Kanter *et al*., 2005; Turkdogan *et al*., 2001; Turkdogan *et al*., 2003), the hepatoprotective effect of *N. sativa* was attributed to the presence of highly potent antioxidants such as thymoquinone, carvacrol, t-anethol and 4-terpineol, phytosterols, phenols and tocopherols, which prevent the transformation of CCl_4 to trichloromethyl free radical and trichloromethyl peroxyl radical (Houghton *et al*., 1995; Ramadan *et al*., 2003; Daba and Abdel-Rahman, 1998; Burits and Bucar, 2000; Dakhakhny *et al*., 2000).

Effect of *T. claveryi* extracts on liver weight/body weight ratio: Figure 1 depicts the effect of *T. claveryi* extracts on Liver Weight/Body Weight Ratio (LW/BW). CCl_4 intoxicated rats developed pronounced hepatomegaly in comparison with the normal control, LW/BW almost doubled in the positive control. This hepatomegaly can be attributed to the action of Constitutive Androstane Receptor (CAR), which is a central regulator of xenobiotic metabolism. CAR activation induces hepatic expression of detoxification enzymes and transporters which increases liver size (Huang *et al*., 2005). The ingestion of *T. claveryi* aqueous extract normalized the effect of CCl_4 on LW/BW ratio, whereas methanolic extract decreased LW/BW ratio significantly while petroleum ether extract was ineffective. This indicates that the quality and quantity of antioxidants in the aqueous extract was superior to that

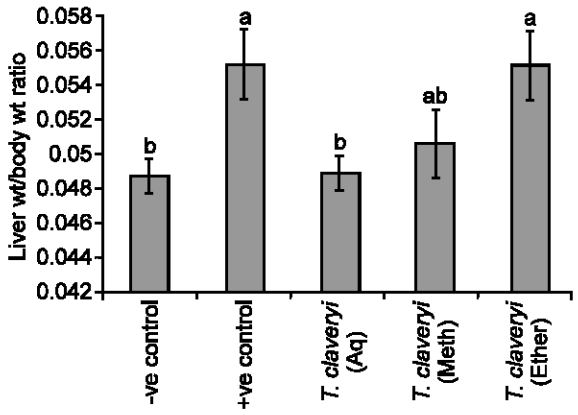

Fig. 1: Effect of *T. claveryi* extracts on Liver weight/body weight ratio. -ve control; Normal rats, +ve control; CCl_4 intoxicated rats. Aq; Aqueous, Meth; Methanolic, Ether; Petroleum ether. Values are expressed as mean±SEM (n = 5). P-values were calculated by Students t-test. Means with different superscripts (a,b) differ significantly (p<0.05)

in the methanolic and petroleum ether extracts, this inhibited the biotransformation and mounting of CCl_4 to $CCl_3^{\cdot}$ and $CCl_3O_2^{\cdot}$ thus decreasing the need for detoxification enzymes and transporters (Recknagel *et al*., 1989; Huang *et al*., 2005).

Effect of *N. sativa* extracts on liver weight/body weight ratio: Figure 2 depicts the effect of *N. sativa* extracts on liver LW/BW ratio. Once again CCl_4 intoxicated rats developed pronounced hepatomegaly in comparison with the normal control which is attributed to the action of CAR which increases the expression of detoxification enzymes and transporters that leads to increased liver size (Huang *et al*., 2005). Aqueous and methanolic

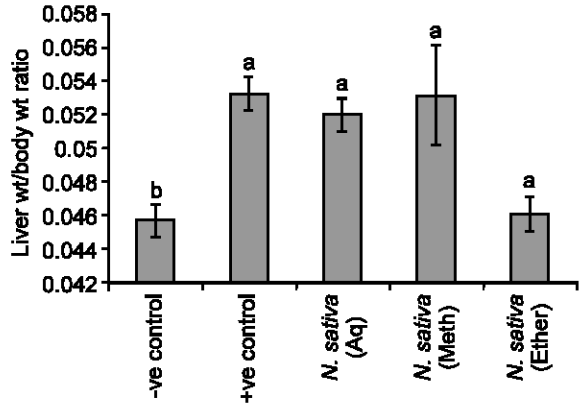

Fig. 2: Effect of *N. sativa* extracts on Liver weight/body weight ratio. -ve control; Normal rats, +ve control; CCl_4 intoxicated rats. Aq; Aqueous, Meth; Methanolic, Ether; Petroleum ether. Values are expressed as mean±SEM (n = 5). P-values were calculated by Students t-test. Means with different superscripts (a,b) differ significantly ($p<0.05$)

extracts of *N. sativa* did not affect the significant increase induced by CCl_4. Whereas, the ingestion of *N. sativa* petroleum ether extract normalized the effect of CCl_4 on liver weight/body weight ratio which indicates the abundance of fat soluble antioxidants such as tocopherols, phytosterols, and phenols in *N. sativa* crude oil plays a major role in the prevention of hepatomegaly (Ramadan *et al*., 2003).

Conclusion: The aqueous extract of *T. claveryi* is as potent as the effect of the reference plant *N. sativa* seeds petroleum ether extract and can be used to prevent liver damage induced by oxidative stress.

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