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Protective Effect of *Nigella sativa* Oil against CCl4-induced Hepatotoxicity in Rats

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الخلاصة:

تم دراسة الدور الذي تلعبه الحبة السوداء في وقاية الكبد من التسمم المستحدث برابع كلوريد الكاربون.

تم تخصيص اربعة مجاميع من جرزان سبراج دولي (Sragueِ Dawley) في كل مجموعة ستة جرذان. اعطيت المجموعة الاولى و الثانية اما 2مل/كغم من محلول الملح الطبيعي او 10 مل/كغم من زيت الحبة السوداء لسبعة ايام ثم تم قتلها في اليوم الثامن بواسطة الاثير ثنائي الاثيل.اما المجموعة الثالثة والرابعة فقد اعطيت اما 2مل/كغم من محلول الملح الطبيعي او 10 مل/كغم/يوم من زيت الحبة السوداء لمدة سبعة ايام وقبل اعطاءها الجرعة الاخيرة بساعة واحدة في اليوم السابع فقد حقنت بـ 2مل/كغم من رابع كلوريد الكاربون (خليط بنسبة 1:1 من رابع كلوريد الكاربون 99% وزيت الذرة) ثم تم قتلها في اليوم الثامن بواسطة الايثر ثنائي الاثيل.

أُخذت عينات من دم قلب الجرذان لتحضير مصل لقياس مستوى الـ (بيليروبين) وفعالية الـ (الينين امينوترانزفيرز). أخذت عينات من نسيج المتجانس في الكبد لغرض قياس (مالونالديهايد) والـ (كلاتاثايون) كما تم تحضيرمقاطع نسيجية من الكبد للفحوصات النسيجية.

اظهرت النتائج ان زيت الحبة السوداء يخفض بشكل ملحوظ انزيمات الكبد المرتفعة في مصل الدم ويثبت حالة الاكسدة الحاصلة بسبب رابع كلوريد الكاربون.

نستنتج من ذلك ان زيت الحبة السوداء يساعد الجرذان في حمايتها من تسمم الكبد الحاصل بسبب رابع كلوريد الكاربون.

Abstract:

The role of *Nigella sativa* L. (Ranunculaceae) (NS) was investigated in the prevention of carbon tetrachloride (CCl_4)-induced liver toxicity.

Twenty four Sprague-Dawley rats were allocated into four groups (6 rats each), first and second groups received either 2m/kg normal saline

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or 10 ml/kg/day NS oil for seven days and sacrificed at day 8 with diethyl ether; third and fourth groups received either 2ml/kg normal saline or 10 ml/kg/day NS oil for seven days for 7 days, and on day 7 they received 2ml/kg CCL_4 (1:1v/v mixture of CCL_4 99% and corn oil)1 hour prior to the last dose, then sacrificed with diethyl ether on day eight. Blood samples were obtained from the hearts to prepare serum for estimation of bilirubin, activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP). Liver tissue homogenate was prepared and used for estimation of malondialdehyde (MDA) and glutathione (GSH), and liver tissue sections were prepared for histological examinations.

The results indicated that NS oil decreased significantly the elevated serum levels of liver enzymes and improve the state of oxidative stress induced by CCl_4.

In conclusion, NS oil protects rats against CCl_4-induced hepatotoxicity.

Key words: Nigella sativa oil, liver toxicity, oxidative stress

Introduction:

Carbon tetrachloride (CCl_4) is a selective hepatotoxic chemical agent, and considered as one of the oldest and most widely used toxins for experimental induction of liver damage in laboratory animals [1]. Free radicals liberated during its metabolic biotransformation in the liver initiate cell damage through two different mechanisms, covalent binding to the membrane proteins and induction of lipid peroxidation [2]. Reactive oxygen metabolites may play an important role in the inflammation process after intoxication by CCl_4[3]. Generation of free radicals and reactive oxygen species (ROS) during metabolic conversion of CCl_4 has been associated with, or contributes to human disease states such as inflammatory diseases and neurodegenerative aging [4,5]. Meanwhile, the associated oxidative stress augment lipid peroxidation through induction of iron overload [6], which may lead to cholestatic injury [7] , liver fibrosis and cirrhosis [8]. The seeds of *Nigella sativa* (NS), sometimes known as black seed, black cumin or Habatul Barakah have long been used in the Middle East as a traditional medicine for a variety of complaints, headache, cough, flatulence, as a choleretic, antispasmodic and uricosuric[9-11]. *N. Sativa* seeds contain 36%–38% fixed oils, proteins, alkaloids, and saponin and 0.4%–2.5% essential oil [10]. The fixed oil is composed mainly of unsaturated fatty acids, including the unusual C20:2 arachidic and eicosadienoic acids [12]. Many other compounds were characterized, but the major ones are thymoquinone (27.8%–57.0%), ρ-cymene (7.1%–15.5%), carvacrol (5.8% 11.6%), t-anethole (0.25%–

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2.3%), 4-terpineol (2.0%–6.6%) and longifoline (1.0%–8.0%) [13]. The present study was designed to evaluate the hepatoprotective activity of *Nigella sativa* oil against CCl_4-induced hepatotoxicity in rats.

Materials and Methods:

Animals:

Twenty male Sprague-Dawley rats (180-220gm) were purchased from the animal house of the College of Pharmacy, University of Baghdad, housed over sawdust beds and maintained on a 12-hr light/dark cycle in a humidity- and temperature-controlled facility at the animal house of the College of Veterinary Medicine, University of Sulaimani; they were allowed to feed of commercial rat pellets and tap water *ad libitum.*

Experimental procedure:

Rats were randomly allocated into 4 groups, first and second groups received either 2ml/kg/day normal saline or 10 ml/kg/day NS oil respectively for 7 days and sacrificed at day 8 with diethyl ether; third and fourth groups received either 2ml/kg/day normal saline or 10 ml/kg/day NS oil respectively for seven days for 7 days, and on day 7 they received 2ml/kg CCL4 (1:1v/v mixture of CCL4 99% and corn oil)1 hour prior to the last dose, then sacrificed with diethyl ether on day 8. Blood samples were collected by cardiac puncture to prepare serum for the estimation of bilirubin [14], activities of alanine aminotransferase (ALT) [15], aspartate aminotransferase (AST) [15] and alkaline phosphatase (ALP) [16]. Liver tissue homogenate was prepared and used for the estimation of malondialdehyde (MDA) [17] and glutathione (GSH) [18]. Moreover, liver sections were prepared and stained with hematoxylin and eiosine for microscopical evaluation of histological changes [19]. The data were expressed as mean ± SEM and statistical analysis was performed using analysis of variance (ANOVA) followed by Student's *t*-test with $P<0.05$ being considered as statistically significant.

Results:

In table-1, administration of CCl4 significantly elevates ALT, AST and ALP activities (516%, 190% and 179% respectively, $P<0.05$) compared to controls; while animals treated with NS oil only demonstrated significant decrease in AST and ALP activities compared to control animals. Table-1 also showed that pre-treatment of rats with NS oil for 7 days, before administration of CCl4, resulted in significant decrease in serum activities of ALT, AST and ALP (69%, 58% and 78% respectively, $P<0.05$) compared to animal group pre-treated with saline

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and challenged with CCl4, and only AST value was found comparable to that of controls.

In table-2, administration of CCl4 significantly elevates serum bilirubin and liver tissue homogenate MDA levels (107% and 192% respectively, $P<0.05$), while GSH level in liver tissue was significantly decreased (57%, $P<0.05$) compared to controls. Meanwhile, administration of NS oil only results in significant elevation of MDA level (38%) and depletion of GSH level (33%) compared to controls, while serum bilirubin was not significantly changed. Table-2 also showed that pre-treatment of rats with NS oil before administration of CCl4 results in significant decrease in serum bilirubin and Liver MDA levels (50% and 57% respectively, $P<0.05$) compared to pre-treatment with normal saline, and also significantly elevates liver GSH level (73%, $P<0.05$) compared to animal group pre-treated with saline; at this stage the oxidative stress parameters are still significantly different compared to that of control animals.

In figure-1, no histological changes were reported due to administration of *N. sativa* oil alone, while pre-treatment with the oil before induction of hepatotoxicity with CCl4 (figure-2) resulted in marked decrease in the histopathological changes already induced by CCl_4, manifested as hydropic degeneration and necrosis (figure-3).

Discussion:

In recent years many studies have shown that various types of plant extracts have wide range of pharmacological activities due to the properties of their constituents [20]. In particular, they contain many types of polyhydroxy-compounds which can function as natural antioxidants in humans and animals. As in other plants, *N. sativa* contain many active constituents that known to have marked antioxidant and reactive oxygen species scavenging potency [21]. To study the hepatoprotective effect of NS oil we used the well described CCl_4-model of rat liver damage [22], in which the liver microsomal oxidizing system connected with cytochrome P-450 produce reactive metabolites of CCl_4 such as trichloromethyl radical ($CCL_3^{\bullet}$) or trichloroperoxyl radical ($CCl_3O_3^{\bullet}$). These radicals cause lipid peroxidation which produces hepatocellular and cholestatic damage and liberate liver enzymes and bilirubin in blood circulation, associated with depletion of GSH and elevation of MDA in liver tissue. In line with this assumption, the present study revealed that pretreatment of rats with NS oil before administration of CCl_4 protected them from the liver damage.

Nowadays lipid peroxidation (LP) is accepted widely as being responsible for the pathogenesis of liver damage. LP aldehydic products

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(malondialdehyde and 4- hydroxynenal) are increased in chronic liver diseases, especially due to alcohol consumption. Lipid peroxyl radicals induce procollagen type I synthesis in stellate cells and so initiate the fibrogenetic process in the liver [23]. Carbon tetrachloride (CCl_4) is a prototype chemical inducing LP in experimental animals within a few minutes of administration. Its long-term use results in liver fibrosis and cirrhosis by the LP pathway [24]. Several plant derived compounds such as colchicine (*Colchicum dispert*), silymarin (*Silybum marianum*), polyenylphosphatidylcholine (soy bean), ellagic acid (cruciferous vegetables), *Gingko biloba* composita and recently, Sho-saiko-to (extract of seven herbs in Chinese folk medicine) have been proposed as antioxidant and antifibrotic in the treatment of chronic liver disease [25,26].

In the present study, the protective effects of *Nigella sativa* was studied in the prevention of carbon tetrachloride induced hepatotoxicity in rats. It was reported that the fixed oil of NS and the derived thymoquinone inhibited eiconasoid generation in leukocytes and membrane lipid peroxidation [27]. Recently, a significant hepatoprotective effect of NS was observed in the histopathological examination of CCl4 administered rabbits. Hepatocellular degenerative and necrotic changes were slight without advanced fibrosis and cirrhotic process in the NS treated group [28]. Thymoquinone, the main ingredient of NS oil, has recently been reported to exhibit hepatoprotective activity possibly by antioxidant and immunostimulant effects [29]. In conclusion, this study suggests new treatments in the prevention of carbon tetrachloride induced hepatotoxicity and reveals the importance of scientific research on miscellaneous plants with various medical properties.

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Treatment groups n=6	Serum ALT Unit/L	Serum AST Unit/L	Serum ALP Unit/L
Normal saline	12.2 ± 0.46 [a]	5.3 ± 0.48 [a]	44.4 ± 2.6 [a]
***N. Sativa* oil + saline**	13.6 ± 0.36 [a]	3.1 ± 0.36 [b]	33.78 ± 2.8 [b]
Normal saline + CCl_4	75.2 ± 7.8 [b]	15.4 ± 0.72 [c]	123.8 ± 12.6 [c]
***N. Sativa oil* + CCl_4**	23.5 ± 1.7 [c]	6.4 ± 1.2 [a]	27.8 ± 3.2 [b]

Table-1: Effect of treatment with *Nigella sativa* oil on the activities of liver enzymes GOT, GPT and ALP in rats intoxicated with CCl_4.

Values presented as Mean ± SE; n= number of animals; values with non-identical superscripts (a,b,c) are considered significantly different (*P*<0.05).

Treatment groups n=6	Serum bilirubin mg/dl	Liver GSH µg/L	Liver MDA µg/L
Normal saline	0.29 ± 0.01 [a]	28.2 ± 1.8 [a]	130.7 ± 15.2 [a]
***N. Sativa oil* + saline**	0.27 ± 0.01 [a]	18.9 ± 0.62 [b]	180.8 ± 22.1 [b]
Normal saline + CCl_4	0.60 ± 0.12 [b]	12.1 ± 0.47 [c]	381.1 ± 10.1 [c]
***N. Sativa oil* + CCl_4**	0.30 ± 0.08 [a]	20.9 ± 0.45 [b]	165.3 ± 18.9 [b]

Table-2: Effect of treatment with *Nigella sativa* oil on the serum levels of bilirubin, glutathione (GSH) and malondialdehyde (MDA) in rats intoxicated with CCl_4.

Values presented as Mean ± SE; n= number of animals; values with non-identical superscripts (a,b,c) are considered significantly different (*P*<0.05).

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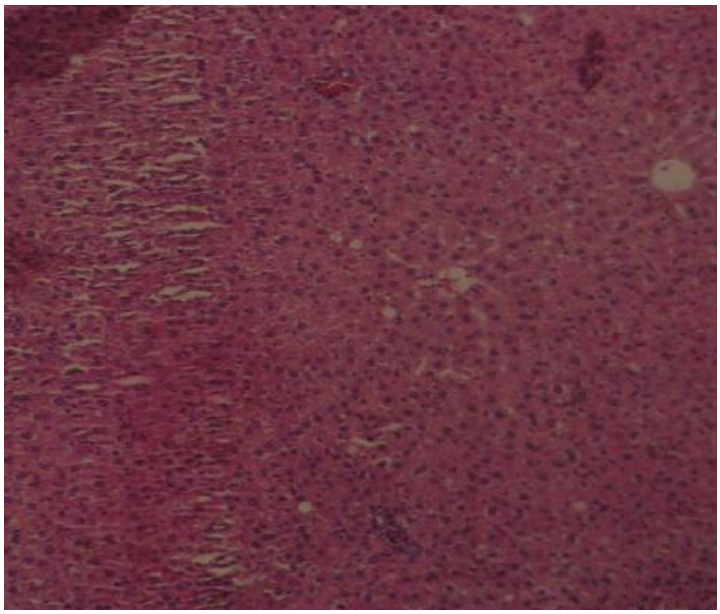

Figure-1: Liver section of rats treated with NS oil. Normal appearance of liver parynchyma and sinusoidal area. HE X 80.

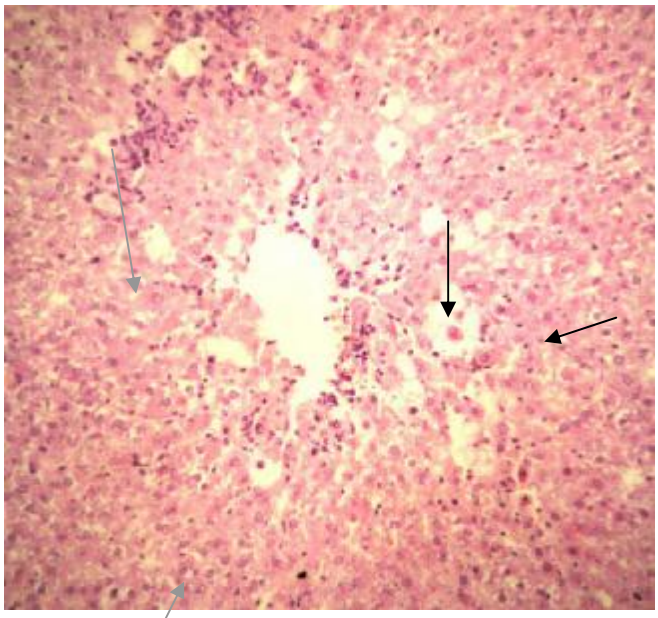

Figure-2: Liver section of rat treated with CCl_4 and saline. Hydropic degeneration (black arrow) and coagulation necrosis (gray arrow) in the hepatocytes and the centrilobular zone (control group). HE X 80.

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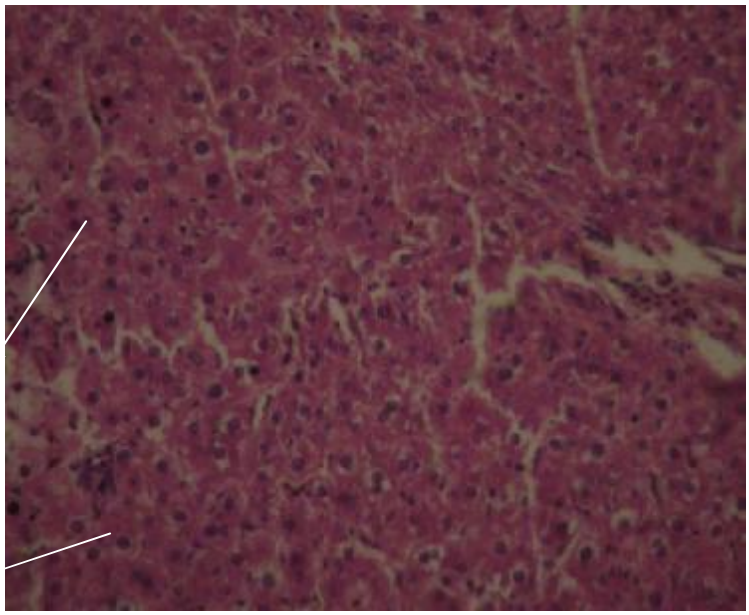

Figure-3: Liver section of rat pre-treated with NS before CCl_4. Normal appearance of liver parenchyma with sparse coagulation necrosis (white arrow). HE X 200.