Protective Effect of Crude Oil of *Nigella Sativa* on Liver in Male Albino Mice Treated with Low Toxic Dose of Paracetamol

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Abstract

Background and purpose of study: Paracetamol when used in very high dose ,can cause heavy damage in liver in man and animals. Nigella sativa has been reported to have antioxidant and hepatoprotective properties. In this study, the effective oral administration of the crude oil of Nigella sativa 0.3 ml ,has been investigated in mice following intra peritoneal of low toxic dose of paracetamol (300 mg/kg B. W.) and measured of selective parameters indicative of liver function (serum GOT, GPT and total protein).

Material and Method :20 adult male albino mice Balb/ C they were randomly divided into 4 groups .The first group injected intra peritoneal with low toxic dose of paracetamol 300mg/ kg body weight then administration orally with 0.3 ml of normal saline (0.9 %w/v).The second group was the control injection intra peritoneal with 0.3 ml of normal saline then administration orally with 0.3 ml of normal saline (0.9 %w/v) .Third group administration orally with 0.3 ml of crude oil of Nigella sativa then injected intra peritoneal with 0.3 ml of normal saline (0.9 %w/v) .Fourth group injected intra peritoneal with low toxic dose of paracetamol 300mg/ kg body weight then administration orally with 0.3 ml of 0.3 ml of crude oil of Nigella sativa.

After 24 hr all animals were sacrificed after weighing then take the blood samples by heart puncture for biochemical test and liver were removed out for weighing.

Results and statistical analysis: Showed a significant increase ($p< 0.05$) in the level of serum enzymes in the first group(p) GOT ($110.1\pm0.61^{*}$ I U/L) and GPT($80.2 \pm 26.3^{*}$ IU/L) and also the fourth group(P&N) GOT ($101.60\pm4.34^{*}$I U/L) and GPT($72.8\pm23.88^{*}$ IU/L) and significant increase in total protein ($7.322\pm2.89^{*}$ gm/dl) in third group(N) and fourth group(P&N) ($7.334\pm0.77^{*}$ gm/dl) at $p<0.05$ as compared with control.

Also there is a significant decrease ($p>o.o1$) in body weight ($28.15 \pm7.084^{**}$) and liver weight($1.7837\pm0.99^{*)}$ at ($p>o.o5$).

Key Word: paracetamol, *Nigella sativa*, mice.

التأثير الوقائي لزيت الحبة السوداء الخام التجاري على كبد ذكور الفئران البيض المعاملة بالجرعة السامة الواطئة للبراسيتامول

الخلاصة

يمكن ان تسبب الجرعة السامة من عقار الباراسيتامول ضررا في كبد الانسان او الحيوان ، لذا استخدمت الحبة السوداء التي لها صفات مضادة للاكسدة وحماية الكبد. لقد استخدمت في هذه الدراسة الزيت الخام للحبة السوداء التجاري عن طريق الفم وبجرعة ٠,٣ مل وتم حقن الحيوانات بالجرعة السامة الواطئة ٣٠٠ ملغم / كغم من وزن الجسم من عقار الباراسيتول داخل البريتون . وقيست مستويات انزيمات الكبد في المصل GPT،GOT والبروتين الكلي . قسمت ٢٠ من ذكور الفئران البيض من طراز بالب / سي عشوائيا الى اربع مجاميع المجموعة الاولى حقنت داخل البريتون بالجرعة السامة الواطئة من عقار البراسيتول ٣٠٠ملغم /كغم من وزن الجسم ثم جرعت ب ٠,٣ مل من محلول الملح الوظيفي (٠,٩ %غم /لتر)عن طريق الفم . المجموعة الثانية اعتبرت مجموعة سيطرة حيث حقنت داخل البريتون ب ٠,٣ مل من محلول ملحي ثم جرعت فمويا ب ٠,٣ مل من محلول الملح الوظيفي (٠,٩ %غم

/لتر)عن طريق الفم .جرعت المجموعة الثالثة عن طريق الفم ب ٠,٣ مل من زيت الحبة السوداء وحقنت داخل البريتون ب ٠,٣ مل من محلول الملح الوظيفي (٠,٩ %غم /لتر). المجموعة الرابعة حقنت داخل البريتون بالجرعة السامة الواطئة من عقار البراسيتول ٣٠٠ملغم /كغم من وزن الجسم ثم جرعت عن طريق الفم ب ٠,٣ مل من زيت الحبة السوداء .بعد ٢٤ ساعة من التجربة تم التضحية بجميع الحيوانات بعد وزنها وجمعت عينات الدم عن طريق ثقب القلب لاجراء الاختبارات البايوكيميائية واخذ الكبد للوزن.

اظهر التحليل الإحصائي زيادة معنوية ($p< 0.05$) في مستويات انزيمات المصل في المجموعة الاولى 110.1±0.61) GOT (p) (P&N) GOT (101.60±4.34*I U/L) وكذلك المجموعة الرابعة GPT(80.2 ±26.3* IU/L) و I U/L) * و GPTوزيادة معنوية في قيمة البروتين الكلي (gm/dl * 7.322±2.89) في المجموعة الثالثة(N) و IU/L) *72.8±23.88) المجموعة الرابعة (gm/dl * 7.334±0.77) (P&N)عند p<0.05 مقارنة مع مجموعة السيطرة . كذلك هنالك انخفاض معنوي (p>o.o1) في وزن الجسم (**7.084± 28.15) ووزن الكبد (*)1.7837±0.99)عند p>o.o5) .

Introduction

Nigella sativa Linn., is an annual herb that belongs to the botanical family of Ranuculaceae, commonly known as the black cumin seed, and there are many common terms from Roman, Russia, India and Pakistan. It is one of important medicins in Tibbe Nabawi i.e. ,Prophet medicine [1]. In Muhammad 's time (the prophet of God) was referred to(Habbat al Baraka) and he was said about it ((Use this black seed regularly it is a cure for every disease ,except death)) [2,3]. It has been employed for thousands of years as spice and food preservative, as well as a protective and curative remedy for numerous disorders. It is known to have many medicinal properties in traditional medicine [4,5]. There are a wide range of studies which proved hepato-protective effect of *Nigella sativa* [6,7] as well as its mild hepatotoxicity in animals [8].

It is produced as hepatoprotective agents effect in some models of liver toxicity [9,10] and there are some scientific research use chemicals as hepatotoxins (toxic to the liver) showed the protective role of *Nigella sativa* (black seed) in modulating the toxic effects induced by these additives [11,12].

Thymoquinone is the bioactive and the most abundant constituent of the volatile oil of this seed which has been shown to possess therapeutic effects, including anti-inflammatory ,antimicrobial, anticancer, antihyp-ertensive, anti-diabetic, and anti-oxidants agent [13]. Thymoquinone is the major active principle of *Nigella sativa* and most of its pharmacodynamic effects are due to thymoquinone,it were found to be highly bio available providing significantly greater protection against free radical-induced lipid per oxidation and DNA damage [14,15].

The present study was designed to find out the role phytomedicinal properties of oil of *Nigella sativa* on some liver enzyme level and total protein of mice treated with the low toxic dose of paracetamol.

Paracetamol is used to treat many conditions such as headache, muscle aches, arthritis, backache, toothaches, colds, and fevers. It relieves pain in mild arthritis but has no effect on the underlying inflammation and swelling of the joint [16].

Paracetamol or acetaminophen , chemically named N-acetyl-p-aminophenol (APAP) [17].

While generally safe for use at recommended doses (1,000 mg per single dose and up to 4,000 mg per day for adults)[18]. The initial symptoms of overdose are nausea ,vomitimg ,diarrh and abdominal pain [19] acute overdoses of paracetamol can cause potentially fatal kidney, brain and liver damage and, in rare

individuals, a normal dose can do the same[20]. Paracetamol toxicity is one of the leading causes of liver failure in the USA accounting for more than 56,000 ER and deaths per years [21].

In cases of paracetamol overdose, the sulfate and glucuronide pathways become saturated, and more paracetamol is shunted to the cytochrome P450 system to produce NAPQI. As a result, hepatocellular supplies of glutathione become depleted, as the demand for glutathione is higher than its regeneration[22]. NAPQI therefore remains in its toxic form in the liver and reacts with cellular membrane molecules, resulting in widespread hepatocyte damage and death, leading to acute hepatic necrosis [23].

Materials and Methods

Animals

Twenty adult male albino mice ,weighing (25-36 gm) were obtained from Iraqi Center for Drug Research/Baghdad, were housed in metabolic cages under controlled environmental conditions (25 ºC and 12h light⁄ dark cycle) animals feed a standard pellet food and tap water.

Crude *Nigella sativa* oil

Crude oil were purchased traditionally from the local market in Hilla city

Material ,this oil was administered orally to mice animal feeding intubations needles (0.3ml from Nigella sativa oil) [24] .

Paracetamol

375 mg/ 5 ml ampoule, the lethal dose of paracetamol (1g/kg/bw) (in this experiment used sublethal 300 mg/kg (body weight)prepared by :Dr. Azar Abdul-Hafudh (M.Sc.Pharmacology), Dentistry college University of Babylon)[25].

Experimental Design

Mice were randomly divided into 4 groups. each consisting of 5 animals. All animals were fasted over night before the experiment :-

Group 1 : Mice received single dose of (300mg /kg) B .W of paracetamol by injected intraperitoneal followed by 0.3 ml of (0.9% w/v) normal saline orally.

Group 2: Mice received 0.3 ml (0.9% w/v) normal saline by injected intraperitoneal then administered 0.3 ml of normal saline orally (control).

Group 3: Mice received 0.3 ml (0.9% w/v) normal saline by injected intra peritoneal then administered 0.3 ml of crude oil of *Nigella sativa* orally .

Group 4 : Mice received single dose of (300mg /kg) B .W of paracetamol by injected intraperitoneal then administered 0.3 ml of crude oil of *Nigella sativa* orally.

Twenty four hour after of treatment ,all mice sacrificed and dissected .The liver were take out and weighed on electronic balance.

Biochemical tests

Blood were drawn via cardiac puncture technique from anesthetized mice and were then centrifuged at 3000 rpm for 10 min to separate the serum. The serum was stored at -40°C until enzyme assays were carried out. The hepatic enzymes alanine aminotransferase (ALT or GPT) activities , alkaline phosphates (AST or GOT) activities were measured by (using Roche Liver Enzyme Kits based on the Randox company) and serum total protein concentration [26].

Statistical analysis

The values are presented as mean ± SD standard deviation .Differences between group means were estimated using a one way ANOVA. The significancy was tasted by finding LSD [27].

Results

Biochemical tests

Paracetamol (300mg /kg) given intraperitoneal showed hepatotoxicity

after 24 has evident from biochemical parameter of study. The levels of liver enzymes in mice group injection with low toxic dose of paracetamol (P) GOT (110.1±0.61 * I U/L) and GPT(80.2 ±26.3* IU/L) and also the group of mice that take paracetamol and crude oil of *Nigella sativa* (P&N) GOT (101.60±4.34*I U/L) and GPT(72.8±23.88* IU/L) revealed significant increase at $p<0.05$ as compared with control (table 1).

Also not the significant increase in total protein (7.322±2.89* gm/dl) in group of mice that administered *nigella sativa* only and group that received paracetamol and crude oil of *Nigella sativa* (P&N) *(*7.334±0.77* gm/dl) at $p<0.05$ as compared with control.

The weight of body and the liver

The results in (table 2) show significant decrease at $p>0.01$(28.15 ±7.084**) in group of mice that received paracetamol and crude oil of *Nigella sativa* (P&N) together only compared with three group .the weight of liver never be affected in all treatment in present study.

*Significant as compare to control group at P> 0.05 (F table value 3.15).

**Highly significant as compare to control group at P> 0.01 (F table value 4.34).

Table 1 The effect of the injection of (300mg/kg) paracetamol and administration of crude oil of *Nigella sativa* (alone and together)on liver enzymes level and total protein in albino mice.

Parameter / Treatment	S GOT(AST) IU/L	S GPT (ALT) IU/L	Total protein gm/dl
Paracetamol (P) (300mg/kg)	110.1±0.61*	80.2 ±26.3*	5.184±1.89
Control	89.33±9.50	45.8±22.72	5.34±4.09
***Nigella sativa*(0.3 ml of crude oil) (N)**	92.3±1.67	49.2±22.72	7.322±2.89*
Paracetamol (300mg/kg) & *Nigella sativa*(0.3 ml of crude oil) (P&N)	101.60±4.34*	72.8±23.88*	7.334±0.77*
F calculated value	6.23	6.557	3.98
LSD value	8.33	9.428	0.977

P = Paracetamol N = *Nigella sativa*

Value expressed as mean ± S.D.

Table 2 The effect of the injection of (300mg/kg) paracetamol and administration of crude oil of *Nigella sativa* (alone and together)on the weight of body and the liver in albino mice.

Parameter / Treatment	Body weight/gm	Liver weight/gm
Paracetamol (P) (300mg/kg)	29.12±5.514	1.98 ±0.46
Control	31.47 ± 4.433	2.126 ±0.51
***Nigella sativa*(0.3 ml of crude oil) (N)**	29.42 ± 3.528	1.9327±0.66
Paracetamol (300mg/kg) & *Nigella sativa*(0.3 ml of crude oil) (P&N)	28.15 ±7.084**	1.7837±0.99
F calculated value	0.9537	21.998
LSD value	4.094	0.2465

P = Paracetamol N = *Nigella sativa*
Value expressed as mean ± S.D.

Discussion

The present study was designed to investigate protective effect of *Nigella sativa* oil on liver function against paracetamol induced acute toxicity ,paracetamol hepatotoxicity manifested biochemically by elevation of serum levels of liver enzyme [28,29]. Paracetamol caused a significant increase in serum GPT and total protein compared with results [30].

The significantly increase of Alanine aminotransferase (ALT or GPT), Aspartate aminotransferase (AST or GOT) enzymes in group treated with paracetamol indicated to the parenchymal hepatotoxic effect induced by paracetamol [31].This increase maybe cause by the damage of liver cell membrance by free radicals and release of these enzyme into blood [32].

These enzymes considered as indicators of functional disorders in livers especially the enzyme GPT [33,34] these enzymes which are normally located in the cytosol are released into the blood flow if the liver injury or cell death enzyme escapes from the cytosol leading to a rise in the serum level of these enzymes [35] .

[36,37] found *Nigella sativa* effectively improve return the liver function by lowering the activities of ALT, ALP.

The study of [30] the liver histopathology showed marker reduction in sinusoidal dilation ,midzonal necrosis after treatment with *Nigella sativa* extract and this indicator to hepatoprotective action against paracetamol These results due to the time that need for the liver to pretreatment and return to his function again .In present study the level of liver enzyme stile significantly increased after treatment with *Nigella sativa* because the period of treatment only one day which mean it need more time to return the function.

The slowdown of body weight in *Nigella sativa* treated mice might be related to the liver enzyme (ALT and AST) level decrease, possibly effect of *Nigella sativa* treatment [38].

Study of [39] showed that feed bird offered the diet containing flavomycin or thyme essential oil (black seed) exhibited increased in body weight

and this may be that use of *Nigella sativa* oil directly inhibits the electrogenic intestinal absorption of glucose and these result agree with [40].

The first demonstration that *Nigella sativa* (black seed) directly inhibits the electrogenic intestinal absorption of glucose in vitro. Together with the observed improvement of glucose tolerance and body weight in rats after chronic oral administration in vivo. Several studies have reported hepatoprotective activity of *Nigella sativa* and its active constituent thymoquinone ,[41]tested the essential oil of *Nigella sativa* for antioxidant activity and showed that thymoquinone carvacrol, t-anethole and 4-terpineol high free radical scavenging property . These results proved that TQ is associated with beneficial changes in hepatic enzyme activities and thereby exerts potential anti-hyperglycemic effects[42] .

N. sativa is of immense therapeutic benefit in diabetic individuals and those with glucose intolerance as it accentuates glucose-induced secretion of insulin besides having a negative impact on glucose absorption from the intestinal mucosa[43] .

Conclusion

From the results of this study it can be conclude that treatment with low lethal dose of paracetamol caused damage to the liver but when administered the crude oil of *Nigella sativa* after injection with paracetamol may be protective the liver but it need more time than one day.

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