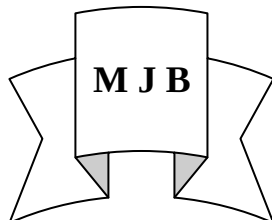


The Phytotherapeutic Effect of Traditional Crude Oil of *Nigella sativa* on Male Reproductive System of Albino Mice Treated with Low Toxic Dose of Paracetamol

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Abstract

The study included (20) adult male Swiss albino mice Balb/ C, they were randomly divided into four groups: The first was injected intra peritoneal with 300 mg / kg body weight (B.W.) of paracetamol which considered as low toxic dose, followed by 0.3 ml of normal saline (0.9 % w/v) was administrated orally . Second, mice administrated orally with 0.3 ml of crude traditional oil of *Nigella sativa*, then injected intra peritoneal with 0.3 ml of normal saline (0.9 % w/v). Third , mice injected intra peritoneal with 300 mg / kg (B.W.) of paracetamol , then administrated orally with 0.3 ml of crude traditional oil of *Nigella sativa* . Fourth the control group, mice injected intra peritoneal with 0.3 ml of normal saline (0.9 % w/v), then administrated orally again with 0.3 ml of normal saline.

The experimental time of treatment were 24 hours for all groups , then all animals were sacrificed, the reproductive organs were dissected out for weighing then preserved in Bouins fixative for histopathy.

Statistical analysis showed a significant increase ($P < 0.05$) in the value of GPT (0.043 ± 0.017) /IU , in the mice treated with 300 mg /kg (B.W) of low toxic dose of paracetamol in compare with control and other treated groups . Also, there is a significant increase ($P < 0.05$) in the weight of seminal vesicle (0.213 ± 0.058) /gm in mice administrated with 0.3 ml of crude oil of *Nigella sativa* as compare with control and other treated groups .

Mice group administered with 0.3 ml of crude oil *Negilla sativa* revealed highly significant increase in sperm count ($336 \pm 100.17 \times 10^4$) sperm /mm³ at ($P < 0.01$) as compared with control and other treated groups

Histometry of reproductive organs, revealed a significant increase ($P < 0.05$) in the wall thickness of testicular seminiferous tubules (66 ± 26.8) / μ in mice administrated with 0.3 ml of crude oil *Negilla sativa* , in contrast to control group and other treatments .

Whereas ,the diameter of seminiferous tubules and the height of glandular epithelia of seminal vesicle never be affected either by the administration of crude oil of *Negilla sativa* nor with the injection of 300 mg /kg (B.W) of low toxic dose of paracetamol .

The weights of right and left testis and prostate gland never be affected with all treatments that included in the current study.

التأثير العلاجي لزيت الحبة السوداء الخام التجاري على الجهاز التكاثري الذكري في الفئران البيض المعاملة بالجرعة السامة الواطئة للبراسيتامول

الخلاصة

صممت هذه الدراسة لتشمل (٢٠) من ذكور الفئران البيض البالغة طراز بالب / سي والتي قسمت عشوائيا الى اربعة مجاميع ، حققت المجموعة الاولى داخل البريتون بالجرعة السامة الواطئة للبراسيتامول (٣٠٠ ملغم / كغم وعلى اساس وزن الجسم) وجرعت بـ 0.3 مل من المحلول الوظيفي (0.9 % غم/لتر) عن طريق الفم . اعطيت المجموعة الثانية وعن طريق الفم (٠,٣ مل من زيت الحبة السوداء الخام التجاري) وحققت داخل البريتون بـ 0.3 مل من المحلول الوظيفي . اما المجموعة الثالثة فقد حققت داخل البريتون بالجرعة السامة الواطئة للبراسيتامول (٣٠٠ ملغم / كغم) ، ومن ثم اعطيت وعن طريق الفم (٠,٣ مل من زيت الحبة السوداء الخام التجاري) . المجموعة الرابعة (مجموعة السيطرة)، تم حقنها داخل البريتون بالمحلول الوظيفي ومن ثم جرعت وعن

طريق الفم (٠,٣ مل من المحلول الوظيفي) . استمرت فترة المعاملة (٢٤ ساعة) ولكل مجاميع التجربة ، تم بعدها التضحية بالحيوانات واستئصال الاعضاء التكاثرية ووزنها ثم حفظها بالمحلول المثبت لغرض تحضير المقاطع النسيجية واجراء القياسات المجهرية ، فضلا عن حساب العدد الكلي للنطف (في البربخ الايسر) .

اظهر التحليل الاحصائي للنتائج وجود زيادة معنوية ($P<0.05$) في وزن كل من البربخ الايمن والايسر (0.043 ± 0.017 ، 0.006 ± 0.042) / غم وعلى التوالي في الفئران المعاملة بالجرعة السامة الوطنية للبراسيتامول مقارنة بمجموعة السيطرة وباقي المعاملات . اما معاملة الفئران بـ (٠,٣ مل) من زيت الحبة السوداء الخام التجاري فقد ادت الى حدوث زيادة معنوية ($P<0.05$) في وزن الحويصلة المنوية (0.058 ± 0.213) / غم و في سمك جدار النبيب المنوي للخصى (66 ± 26.8 / مايكرون ، فضلا عن ظهور زيادة عالية المعنوية ($P<0.01$) في العدد الكلي للنطف ($100.17 \pm 336 \times 10^4$) نطفة/ملم^٣ ، مقارنة بمجموعة السيطرة وباقي المعاملات .

اما بالنسبة لاوزان الخصى ، وزن غدة البروستات ، اقطار النبيبات المنوية وارتفاع الظهارة المبطنه للحويصلة المنوية فلم تظهر اي تغير معنوي ولجميع المعاملات التي اشتملت عليها الدراسة الحالية .

Introduction

N*igella sativa*, is a genus of about 25 species of small prostrate annual herbaceous , dicotyledons plants in the family Ranunculaceae, native to southern Europe, North Africa and South west Asia. The plant grow to 20-90 cm tall, with finely divided leaves. The leaf segments narrowly linear to thread like, the flowers are delicate with yellow, pink, pale, blue or pale purple, with 5-10 petals, the fruit is a capsule composed of several united follicles each containing numerous seeds [1, 2].

Nigella sativa has been used traditionally for medicinal purposes, both as herb and pressed into oil for a variety of conditions and treatments related to respiratory health, stomach, intestinal health, kidney and liver function [3-5]

Seeds contain numerous esters of structural unusual unsaturated fatty acids with terpenes alcohol; traces of alkaloid are found which belong to two different types: isochinoline alkaloids are represented by nigellimin and nigellimin-N-oxide and pyrazol alkaloids include nigillidin and nigellicin [3].

In the essential oil, thymoquinone was identified as the main component and significant amount of fatty acid ethyl esters. On storage, thymoquinone yields

dithymoquinone and higher digocondensation products. The seeds also contain a fatty oil rich in unsaturated fatty acids (linoleic acid, oleic acid and dihomolinoleic) and saturated fatty acids (palmitic and stearic acid) [6,7].

The aqueous extracts of *Nigella sativa* have increased spermatogenesis of male albino rats at primary and secondary spermatocyte stages the elevation of spermatozoa number as well as the elevation of the secretory activities of accessory glands [8].

Histopathological observations showed that *Nigella sativa* significantly preserved visceral organ architecture changes induced by various toxic chemicals [9], and this autidotes protection have been primarily attributed to their radical scavenger ability and inhibition of oxidative stress induced by different toxic chemicals therefore, *Nigella sativa* seeds were used since ancient time in treating natural killer and in treating patients suffering from liver cirrhosis and hepato cellular damage [10 - 12].

The present study was designed to find out the role of phytotherapeutic agent of *Nigella sativa* on the male reproductive system of mice treated with the low toxic dose of paracetamol.

Paracetamol, is also known as acetaminophen-N-acetyl-p-aminophenol (APAP), is considered to be a safe analgesic and antipyretic agent taken in the therapeutic dose [13]. In overdose, it can cause hepatic and different other organs damage, and this is invariably followed by recovery with the early administration of intravenous N-acetylcysteine or oral methionine (NAC) which considered as antidote [14,15].

The toxic dose of APAP with a single acute ingestion is 150 mg / kg, its rapidly absorbed from the stomach and small intestine and metabolized by conjugation in the liver to nontoxic agents, which are eliminated by urine.

In paracetamol treated mice, shrinkage of the seminiferous tubules and disturbance of regular arrangement of the spermatogenic cells in the testis were observed i.e. the spermatogenic cells were not arranged in contact regular layer as in the case of the normal [16]. Also, [17] studied the long term effect of aspirin on the rat testis and found that aspirin caused significant histological damage and concluded that the disturbed spermatogenesis may be due to the inhibition by aspirin of prostaglandin synthesis that is necessary for the completion of spermatogenesis.

The antidote to APAP poisoning (NAC), a precursor of glutathione, which in addition to increasing glutathione unmetabolized APAP, NAC. Function as an antioxidant and also has indirect vasodilatory effect by increasing local nitric oxide (NO) concentrations. The vasodilatory effect on microcirculation enhances local oxygen delivery to peripheral tissues, and thus decreases morbidity and mortality even in established hepatocyte [18].

Acetaminophen toxicity may result from a single toxic dose, from ingestion of large doses of

acetaminophen (e.g., 7.5 – 10) gm daily for (1-2 day). [19,20]

Paracetamol, is non prescription drug commonly used for antipyresis and analgesic [21]. It contains a phenol ring, as does oestradiol and an acetyl group as does progesterone, these structural features suggest that paracetamol may possess sex steroid agonist or antagonist activity. Indeed, Uterine, ovarian and testicular atrophy has been reported in mice fed with high doses of paracetamol [22] these observations indicate a possible anti gonadotrophic effect of paracetamol. Further, it is now known that paracetamol can inhibit nitric oxide generation [23], which is essential for normal reproductive activity in the male rat [24].

Paracetamol also has a mild inhibitory action on prostaglandin synthesis in peripheral tissues [25]. In high doses (500-1000 mg/kg) impair fertility on male rats [26]. These observations raise the possibility that long-term use of high doses of paracetamol may interrupt normal reproductive function due to its antigonadotrophic activity [27].

Materials and Methods

Animals: (20) adult male Swiss albino mice of Balb/c strain, weighing (27-36 gm) with an age 8 weeks were purchased from Iraqi Center for Drug Research/ Baghdad, these animals were housed during the period of experiment in the animal house unit in medicine college of Babylon University, under controlled temperature of $(21 \pm 1\text{ }^{\circ}\text{C})$ and (12 hours) light, (12 hours) darkness schedule, food and water were available *ad libitum*.

Crude *Nigella sativa* oil : crude oil were purchased traditionally from the local market in Hilla city, this oil was administered orally to mice using animal feeding intubation needles.

Paracetamol (Hyamol) : 375 mg/5 ml ampoule (the experimental dose used in the current study was 300 mg/kg (body weight) prepared by: Dr. Azhar Abdul-Hafudh (M.Sc.

Pharmacology), Dentistry college, Babylon University) .

Experimental Design: - Fasted mice were randomly distributed into four groups, each group contains 5 animals, and as the follows table :-

Groups	Treatments
1	Mice injected intra peritoneal with (300mg/ kg)B.W of paracetamol followed by 0.3 ml of (0.9 % w/v) normal saline orally.
2	Mice injected intra peritoneal with normal saline 0.3 ml , then, administered with 0.3 ml of <i>Nigella sativa</i> crude oil orally
3	Mice injected intra peritoneal with (300 mg / kg) B.W. of paracetamol, then , administered with 0.3 ml of crude oil of <i>Nigella sativa</i> orally .
4	Mice injected intra peritoneal with normal saline 0.3 ml then, administered with 0.3 ml of normal saline orally.

Then all animals were sacrificed under light ether anesthesia after 24 hours of treatment, the reproductive organs were taken out, trimmed free of fat, each organ was weighed separately on electronic balance. The reproductive organs taken into account for study include.

Right and left testis, right and left epididymis, prostate gland and seminal vesicle, all these organs were preserved in Bouins fixative for histological studies and histometry.

Sperm count:- Left epididymis was minced in 2ml of physiological saline then add one drop of eosin (1% solution), the mixed sample was applied to a Neubauer counting chamber under cover slip as the method in [28]

Histometry:- Slides were prepared according to Persnel and Schreebman [29]. Microscopic measurements of seminiferous tubules diameter, thickness of its wall (spermatogenic layer) and the height of seminal vesicle epithelia was done by using the ocular micrometer after its calibration with stage micrometer and for different magnifications.

Statistical analysis:- All data were subjected to a one-way analysis of variance (ANOVA) to determine the level of significance between control and the treated groups. The significance was tested by finding LSD. Data are reported here as mean $\pm$ standard deviation ($\pm$ SD). [30].

Results

1- Weight of reproductive organs :

The results in (table 1) show a significant increase ($P < 0.05$) in the weight of right and left epididymis (0.043 ± 0.017 , 0.042 ± 0.006)/ gm in sequence , in the mice treated with 300 mg /kg (B.W) of low toxic dose of paracetamol in compare with control and other treated groups . Also, there is a significant increase ($P < 0.05$) in the weight of seminal vesicle (0.213 ± 0.058)/gm in mice administrated with 0.3 ml of crude oil of *Nigella sativa* as compare with control and other treated groups .

The weights of right and left testis and ventral prostate never be affected in all treatments that included in the present study.

2- Sperm count:

Mice group administered with 0.3 ml of crude oil *Negilla sativa* revealed highly significant increase in sperm count ($336 \pm 100.17 * 10^4$) sperm /mm³ at (P <0.01) as compared with control and other treated groups (table 1).

3- Histometry of reproductive organs :

Statistical analysis of data in (table 2) revealed a significant increase (P<0.05) in the wall thickness of

testicular seminiferous tubules (66 ± 26.8) / μ in mice administrated with 0.3 ml of crude oil *Negilla sativa*, in contrast to control group and other treatments.

Whereas ,the diameter of seminiferous tubules and the height of glandular epithelia of seminal vesicle never be affected either by the administration of crude oil of *Negilla sativa* nor with the injection of 300 mg /kg (B.W) of low toxic dose of paracetamol .

Table 1 The effect of the injection of (300mg/kg) paracetamol and administration of crude oil of *Negilla sativa* (alone and together) on the weight of male reproductive organs and sperm count in albino mice.

Parameters Treatments	Testis / gm		Epididymis / gm		Prostate / gm	Seminal vesicle /gm	Sperm count * 10 ⁴ (sperm/mm ³)
	Right	Left	Right	Left			
Control	0.047 ± 0.06	0.0701 ± 0.06	0.028 ± 0.026	0.027 ± 0.011	0.057 ± 0.044	0.092 ± 0.06	180 ± 62.6
Paracetamol (300mg/kg)	0.0534 * ± 0.053	0.0728 * ± 0.03	0.043 ± 0.017	0.042 ± 0.006	0.032 ± 0.006	0.093 ± 0.054	240 ± 62.6
Black cumin <i>Negilla sativa</i> (0.3 ml of crude oil)	0.0808 ± 0.03	0.0764 ± 0.05	0.0303 ± 0.006	0.031 ± 0.012	0.0497 ± 0.05	0.213 * ± 0.058	336 ** ± 100.17
Paracetamol (300mg/kg) & <i>Negilla sativa</i> (0.3ml of crude oil)	0.0604 ± 0.05	0.0742 ± 0.04	0.037 ± 0.007	0.029 ± 0.013	0.027 ± 0.008	0.078 ± 0.044	108 ± 59.03
F calculated value	0.409	0.073	3.18	3.45	2.26	3.26	4.35
LSD value	0.065	0.0134	0.014	0.007	0.013	0.105	150.32

Value expressed as mean ± S.D.

* 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 2 The effect of the injection of (300mg/kg) paracetamol and administration of crude oil of *Negilla sativa* (alone and together) on the histometry of male reproductive organs in albino mice.

Parameters Treatments	Testis histometry / μ 10 x magnification		Height of glandular epithelia of Seminal vesicle/ μ 10 x magnification
	Seminiferous tubules diameter	Wall thickness of seminiferous tubules	
Control	147 $\pm$ 60.8	48 $\pm$ 23.26	40 $\pm$ 26.8
Paracetamol (300mg/kg)	190 $\pm$ 44.7	60 $\pm$ 19.68	56 $\pm$ 14.3
Black cumin <i>Negilla sativa</i> (0.3 ml of crude oil)	192 $\pm$ 48.3	66* $\pm$ 26.8	40 $\pm$ 26.8
Paracetamol (300mg/kg) & <i>Negilla sativa</i> (0.3ml of crude oil)	176 $\pm$ 33.9	52 $\pm$ 14.3	34 $\pm$ 12.5
F calculated value	0.037	3.293	3
LSD value	68.03	12.88	16

Value expressed as mean $\pm$ S.D.

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

Discussion

Paracetamol is principally metabolized in the liver through two major hepatic routes: glucouronidation and sulphation, the latter route can be rapidly saturated at doses above therapeutic doses, then elimination by dose – dependent formation of glucouro- and sulpho-conjugate derivatives [19, 20], and this may be the reason of why not all of the organs affected by the treatment of low toxic dose (300 mg/kg) of paracetamol, such as: testis, prostate and seminal vesicle, where they need more time to exposed to the tested dose before the elimination process take place, while in regards to epididymis weight, the results show a significant increase that may be due to the abnormal production of free radicals that attack cell components, then lead to damage of some cell macromolecules, proteins, lipids and nucleic acid which in turn may cause a vacoulation, infiltration and aggregation of fluids and fat, then increasing their weight [31, 32].

Inspite of the structural features of paracetamol which possess the phenol ring and acetyl group that suggested the sex steroidal (oestradiol and progesterone) agonist or antagonist [22].

The results related to sperm count revealed no significant differences and the explanation due to the spermatogenic process duration time which requires (53 days) out of which spermatozoa spends last (6-7 days) in the final transit through epididymis [33], while the drug spend little time then eliminated as derivatives through urine which mean it need more time to exposed to the therapeutic dose of the drug to expressed the steroidal actions before the elimination process take place.

In contrast, the administration of crude oil of *Negilla sativa* causes a significant increase in the weight of seminal vesicle, wall thickness of testicular seminiferous tubules and the sperm number, in spite of short exposed period to the extract, this may explain on the bases of the

activity of oil components on the enzyme of oxidative phosphorylation [34], whereas, [35] confirmed that the antifertility activity of *Negilla sativa* in male rats has been established shown by an inhibition of spermatogenesis and a significant reduction in sialic acid content of the testis , epididymis, seminal vesicle and prostate gland.

These results agree with those of the study of [36,37] whom they found that *Negilla sativa* ingestion produce increase effects on fertility and reproductive system in adult rats raised from the increase in the accessory glands weights and in the pattern of testosterone secretion. The data regards with the histometry of reproductive organs further confirm the androgen dependent increment .

Rajesh and Howard [38] found that, the possible mechanism of the chemopreventive actions of thymoquinone was suggested to be the result of its antioxidant and anti-inflammatory activities coupled with an enhancement of detoxification process which lead to preserve visceral organ architecture changes induced by various toxic chemicals (such as paracetamol that used in present study) and UV-radiation as in histopathological observation [9], this may be a propable reason for the result of mice group that treated with low toxic dose of paracetamol then administrated with crude oil of *Negilla sativa* which its mechanism of action dissimilar to that of most classic cytotoxic chemotherapeutic agents [39]

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