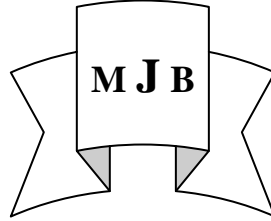


Effect of Crude Oil of Black Seeds (*Nigella sativa*) on White Blood Cell and Hematocrit of Male Albino Mice Treated with Low Toxic Dose of Paracetamol

Zainab Hadi Kamil

Basic Science Department, College of Dentistry, University of Babylon, Hilla, Iraq.



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Abstract

The study was carried out to investigate the effect of crude oil of black seeds (*Nigella sativa*) on white blood cell and hematocrit of mice treated with low toxic dose of paracetamol.

The study included twenty (20) adult male mice Balb/c randomly divided into four groups. Group1 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 . Group2; 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). Group3, 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* . Group4 the control group, mice injected intra peritoneal with 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.

The total leukocyte counts (TLC), differential leucocyte count (DLC) and hematocrit (Hct) was determined.

Statistical analysis of data demonstrated significant increase ($p<0.05$) in TLC, lymphocyte count, neutrophils %, neutrophils count and lymphocyte count of mice treated with *Nigella sativa* crude oil only and with (300 mg/kg) B.W of low toxic dose of paracetamol groups.

Treatment with low toxic paracetamol (300mg/kg) and crude oil of *Nigella sativa* show no significant changes in hematocrit (Hct) value in mice between the four groups.

تأثير زيت بذور الحبة السوداء (*Nigella sativa*) على خلايا الدم البيض ومكداس الدم في

ذكور الفئران البيض المعاملة بالجرعة السامة الواظئة للباراسيتامول

الخلاصة

أجريت هذه الدراسة للتحري عن تأثير زيت بذور الحبة السوداء الخام على خلايا الدم البيض ومكداس الدم في ذكور الفئران البيض المعاملة بالجرعة السامة الواظئة للباراسيتامول. شملت الدراسة ٢٠ من ذكور الفئران البيض البالغة طراز بالب / سي وقسمت عشوائيا الى أربعة مجاميع ، حققت المجموعة الاولى داخل البريتون بالجرعة السامة الواظئة للباراسيتامول (٣٠٠ ملغم / كغم وعلى أساس وزن الجسم) وجرعت بـ 0.3 مل من المحلول الوظيفي (0.9 % غم/لتر) عن طريق الفم. أعطيت المجموعة الثانية وعن طريق الفم (٠,٣ مل من زيت الحبة السوداء الخام) وحققت داخل البريتون بـ 0.3 مل من المحلول الوظيفي. اما المجموعة الثالثة فقد حققت داخل البريتون بالجرعة السامة الواظئة للباراسيتامول (٣٠٠ ملغم / كغم) ، ومن ثم اعطيت وعن طريق الفم (٠,٣ مل من زيت الحبة السوداء الخام). تم حقن المجموعة الرابعة (مجموعة السيطرة) داخل البريتون بالمحلول الوظيفي ومن ثم جرعت وعن طريق الفم (٠,٣ مل من المحلول الوظيفي). استمرت فترة المعاملة ٢٤ ساعة و للمجاميع الأربعة.

بينت النتائج زيادة معنوية ($p < 0.05$) في العدد الكلي لخلايا الدم البيض ونسبة وعدد الخلايا العدلة وعدد الخلايا اللمفية في المجموعة الثانية (الفئران المعاملة بزيت بذور الحبة السوداء والجرعة السامة الواطنة للباراسيتامول) والمجموعة الثالثة (الفئران المعاملة بزيت بذور الحبة السوداء).

لم يظهر لزيت بذور الحبة السوداء والجرعة السامة الواطنة للباراسيتامول أي تأثير في قيم مكاس الدم في ذكور الفئران البيض.

Introduction

Medical plants have been a major source of therapeutic agent since ancient times to cure human disease. The World Health Organization (WHO) estimated that up to 80% of people still rely on herbal remedies for their health care.[1,2] Avicenna in his famous reference "The Canon of medicine refers to *Nigella* as the seed that stimulates the body energy and helps recovery from fatigue and dispiritedness[3].

Of all the plant organs it is only the seeds which attracted most of the researchers[4].

The black seeds were referred to by the prophet Mohammed as having healing powers; Use this Black seeds, it has a cure for every diseases except death[4,6,7]m an identified as the curative black cum in the Holy Bible [6,7].

Most properties of whole seeds or their extracts are mainly attributed to quinone constituents[6]. Pharmacologically the active constituent of the seeds oils includes ; thymoquinone, dithymoquinone, hymoquinone and thymol [8]. Commercial *nigella* oil may also contain parts of the essential oil, mostly thymoquinone by which it acquires an aromatic flavor [7] , possesses several properties including analgesic and anti-inflammatory action [9] and a number of pharmacological effects of profound therapeutic value, like: antihistaminic, anti-allergic, anti-oxidant, anti-cancer, immune stimulation, anti-asthmatic, antihypertensive, hypoglycemic, anti-

bacterial, antifungal, anti-viral and anti-parasitic [4,5,7,10].

The present study was designed to find out the role of phytotherapeutic agent of *Nigella sativa* on the white and red blood cells 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 [11].

Paracetamol shows some life threatening effects like Liver damage which in turn leads to live failure and death. Though it reduces fever and pain, it is found highly toxic. The mechanism by which Paracetamol reduces fever and pain is still not known. On metabolism, Paracetamol is converted to a metabolite which is very toxic to liver cells. In recommended doses (1-2 g/day), Paracetamol does not irritate stomach lining, kidney cells and liver cells [12]. Renal effects of paracetamol overdose are less commonly seen than hepatic effects [13].

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).[14]

Paracetamol reduces the production of prostaglandins and other pro-inflammatory chemicals [12]. Administration of paracetamol increases the bioavailability of serotonin in rats [15].

Materials and Methods

Animals:

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

Crude *Nigella sativa* oil:

Crude oil were purchased traditionally from the local markets in Hilla city , this oil was administered orally to mice using animal feeding intubations needles.

Paracetamol:

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:

Animals were randomly divided into four groups of 5 animals each.

Group 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.

Group 2: Mice injected intra peritoneal with normal saline 0.3 ml , then, administered with 0.3 ml of *Nigella sativa* crude oil orally.

Group 3: Mice injected intra peritoneal with (300 mg / kg) B.W. of paracetamol, then , administered with 0.3 ml of crude oil of *Nigella sativa* orally.

Group 4: Mice injected intra peritoneal with normal saline 0.3 ml then, administered with 0.3 ml of normal saline orally.

Hematological parameters

Blood was collected by cardiac puncture.

The white blood cells (WBC) counts and differential leucocyte count were determined according to Dacie and Lewis [16]. The packed cell volume or hematocrit (Hct) was determined by the microhaematocrit method [17]

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 as mean \pm standard error (\pm SE). [18].

Results

1-Effects on leukocytes count

The results of total leukocyte count (TLC) and differential leukocyte count (DLC) of the four groups are presented in table 1.

Statistical analysis of data demonstrated significant differences ($p < 0.05$) in the values of TLC between the groups. Significant increase ($p < 0.05$) in TLC ($13.48 \pm 3.30 \times 10^3 \mu\text{l}$) of mice administrated with 0.3 ml of *Nigella sativa* crude oil and ($11.36 \pm 0.22 \times 10^3 \mu\text{l}$) of injected with (300 mg/kg) B.W of low toxic dose of paracetamol and administrated with 0.3 ml of crude oil of *Nigella sativa* as compared with control and treated with paracetamol groups.

The results show significant increase ($p < 0.05$) in lymphocyte count ($7.82 \pm 1.91 \times 10^3 \mu\text{l}$ and $6.90 \pm 0.60 \times 10^3 \mu\text{l}$) in mice treated with both low toxic paracetamol and crude oil of *Nigella sativa* group and in mice treated with crude oil of *Nigella sativa* only respectively as compared with other groups.

Significant increase ($p < 0.05$) in neutrophils percentage ($41.4 \pm 1.20\%$ and $40.2 \pm 0.89\%$) in mice treated with both low toxic paracetamol and crude

oil of *Nigella sativa* group and in mice treated with crude oil of *Nigella sativa* only respectively as compared with other groups.

The study shows significant increase ($p < 0.05$) in neutrophils count ($5.49 \pm 1.33 \times 10^3 \mu\text{l}$ and $4.56 \pm 0.62 \times 10^3 \mu\text{l}$) in mice treated with both low toxic paracetamol and crude oil of *Nigella sativa* group and in mice treated with crude oil of *Nigella sativa* only respectively as compared with other groups.

1-Effects on hematocrit

Treatment with low toxic paracetamol (300mg/kg) and crude oil of *Nigella sativa* show no significant changes in hematocrit (Hct) value in mice (table.2)

Discussion

Many studies have been carried out in recent years on the pharmacological effects of black seed oil [4,7,19]. The oil has analgesic, antimicrobial, anti neoplastic, anti-inflammatory and immunological effects [9].

This study demonstrated significant increase ($p < 0.05$) in TLC of mice administrated with 0.3 ml of *Nigella sativa* crude oil only as compared with control and this agree with other studies [7,20,21,22] The observed significant elevation in WBC's count in this study may be due to active materials known as nigellone thymoquinone and thymohydroquinone in *Nigella sativa* oil [23]. Recent study demonstrated also significant increase in TLC of mice injected with (300 mg/kg) B.W of low toxic dose of paracetamol and administrated with 0.3 ml of crude oil of *Nigella sativa* as compared with control or paracetamol only that refers to *Nigella sativa* enhanced immunity of mice treated with paracetamol.

Paracetamol is an effective simple analgesic and antipyretic drug [12,13].

Paracetamol have no effect on TLC and DLC of mice as compared with other groups a similar studied [13,24], which suggested that the immune system have not been compromised [24]

The increasing of TLC of mice treated with *Nigella sativa* is related to the increasing of neutrophils count and percentage in recent study and that is confirmed by other studies [3,7,20]. Neutrophils are increased (neutrophilia) in acute inflammation, and represent the first body defense line [25 textbook physiology]. The acute toxicity of *Nigella sativa* fixed oil was investigated in mice, LD50 values, obtained by single doses, orally and intraperitoneally administered in mice, were 28.8 ml/kg body wt.[19]

Lymphocytes count were significant increase in mice administrated with *Nigella sativa* only as compared with the control and other groups of the study and this result agree with al-Zendi et al.[20] and Al-Attar and Al-Taisan [2]. Lymphocytes represent adaptive immune response [20].

In present experiments the hematocrit Hct was insignificant differentiated of mice administrated with the oil of *Nigella sativa* and the result is agreed with the finding of other study of the effect of *Nigella sativa* oil administration in rats [22] and other study in rabbits [1]. Other studies mentioned significant increase in Hct % of rats treated with black seeds oil and this differences in results is due to the short period of administration (24 hours) of the recent study.

No significant changes is observed in Hct% of mice treated with paracetamol as compared to the control or other groups of the study and this result agree with other studies on paracetamol activity on red blood cells RBC [13,15]. On the other hand Oyedeji et al [24] investigated

treatment of rats for 42 days with 7.5 mg/kg BW of paracetamol caused significant reduction in Hct value, that is paracetamol has potential to inhibit erythropoietin release from the kidneys.

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Table 1 The effect of the injection of (300mg/kg) paracetamol and administration of crude oil of *Nigella sativa* (alone and together) on the total leukocyte count (TLC) and differential leukocyte count (DLC) in albino mice.

Groups WBC count	Groups				F calculated value	LSD value
	Group 1	Group 2	Group 3	Group 4		
TLC (x 10 ³ µl)	7.86 ± 0.17	13.48* ± 3.30	11.36* ± 1.01	6.96 ± 0.22	3.85	4.64
	Lymphocyte (%)	57 ± 1.17	58.2 ± 0.65	60.8* ± 1.08		
No.		4.15 ± 0.15	7.82* ± 1.91	6.90* ± 0.60	4.15 ± 0.10	3.99
Monocytes (%)	2.6 ± 0.97	2 ± 1.1	1 ± 0.61	2.4 ± 1.09	0.17	
	No.	0.18 ± 0.06	1.65 ± 0.16	0.06 ± 0.08		0.17 ± 0.07
Neutrophils (%)	38 ± 5.5	41.4* ± 1.20	40.2* ± 0.89	36.8 ± 1.29	4.08	3.08
	No.	2.98 ± 0.12	5.49* ± 1.33	4.56* ± 0.62		
Eosinophils (%)	1.6 ± 0.75	0.4 ± 0.44	0.1 ± 0.01	1.2 ± 0.65	2.2	1.34
	No.	0.11 ± 0.05	0.06 ± 0.07	0.01 ± 0.01		

Value expressed as mean ± S.E.

* Significant differences at P> 0.05 (F table value 3.15)

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

Groups	Group 1	Group 2	Group 3	Group 4
Hematocrit				
Hct	33.2	34	34.2	33.8
(%)	±	±	±	±
	0.41	0.35	0.42	0.41
F calculated value	1.43		LSD 1.08	

Value expressed as mean ± S.E.