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

Rihab Galib Mohammad AL- Zuhairy

Dep. Animal Production – College of Agriculture - Babylon University

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 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 ($336 \pm 100.17 * 10^4$) sperm /mm^3 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 & 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

رحاب غالب محمد الزهيري

قسم الثروة الحيوانية – كلية الزراعة – جامعة بابل

المستخلص :-

صممت هذه الدراسة لتشمل (20) من ذكور الفئران البيض البالغة طراز بالب / سي و التي قسمت عشوائيا الى اربعة مجاميع ، حقنت المجموعة الاولى داخل البريتون بالجرعة السامة الواطئة للبراسيتامول (300 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 (0.3 مل من زيت الحبة السوداء الخام التجاري) وحقنت داخل البريتون بـ 0.3 مل من المحلول الوظيفي . اما المجموعة الثالثة فقد حقنت داخل البريتون بالجرعة السامة الواطئة للبراسيتامول (300 ملغم / كغم) ، ومن ثم اعطيت وعن طريق الفم (0.3 مل من زيت الحبة السوداء الخام التجاري) . المجموعة الرابعة (مجموعة السيطرة)، تم حقنها داخل البريتون بالمحلول الوظيفي ومن ثم جرعت وعن طريق الفم (0.3 مل من المحلول الوظيفي) . استمرت فترة المعاملة (24 ساعة) ولكل مجاميع التجربة ، ت م 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 ، 0.042 ± 0.006) / غم وعلى التوالي في الفئران المعاملة بالجرعة السامة الواطئة للبراسيتامول مقارنة بمجموعة السيطرة وباقي المعاملات . اما معاملة الفئران بـ (0.3 مل) من زيت الحبة السوداء الخام التجاري فقد ادت الى حدوث زيادة معنوية ($P<0.05$) في وزن الحويصلة المنوية (0.213 ± 0.058) / 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 ($336 \pm 100.17 \times 10^4$) نطفة/ملم^3 ، مقارنة بمجموعة السيطرة وباقي المعاملات .

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

Introduction

Nigella sativa , is a genus of about 25 species of small prostrate annual herbaceous , dicotyledons plants in the family Rannculaceae , 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 releated to respiratory health , stomach, intestinal health, kidney and liver function . (**3** , **4** , **5**)

Seeds contain numerous esters of structural unusual unsaturated fatty acids with terpens 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 & stearic acid) (**6** , **7**)

The aqueous extracts of *Nigella sativa* have increased spermatogenesis of male albino rats at primary & 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 inhaibition 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 phytotherapentic 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 un metabolized 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 deliver to peripheral tissues , and thus decreases morbidity and mortality even in established hepatocyte (**18**) .

Aceptaminophen 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 aceyl 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 observation indicate a possible anti gonadotrophic effect of paracetamol . Further , it is now known that paracetamol come 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 duo to its antigon a dotrophic activity (**27**) .

Materiales & Methods

Animales : (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 ± 1 C$^{\circ}$) and (12 hours) light , (12 hours) darkness schedule , food and water were available *ad libitum*.

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

Parcetamol (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 *Nigella sativa* 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 *Nigella sativa* 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 Neubaures counting chamber under cover slip as the method in (**28**)

Histometry :- Slides were prepared according to Persnel & 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 significancy was tested by finding LSD . Data are reported here as mean ± standard deviation (±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 & 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 & 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 /mm^3 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 & administration of crude oil of *Negilla sativa* (alone and together) on the weight of male reproductive organs & 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 *Negilla sativa* (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) & *Negilla sativa* (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 & 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 ± 60.8	48 ± 23.26	40 ± 26.8
Paracetamol (300mg/kg)	190 ± 44.7	60 ± 19.68	56 ± 14.3
Black cumin *Negilla sativa* (0.3 ml of crude oil)	192 ± 48.3	66* ± 26.8	40 ± 26.8
Paracetamol (300mg/kg) & *Negilla sativa* (0.3ml of crude oil)	176 ± 33.9	52 ± 14.3	34 ± 12.5
F calculated value	0.037	3.293	3
LSD value	68.03	12.88	16

Value expressed as mean ± 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 & 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 & 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 & 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 which spermatozoa spends last (6-7days) 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 & Howard (**38**) found that , the possible mechanism of the chemopreventive actions of thymoquinon 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**)

References

1- Varghese, E. SVD. (1996). Applied Ethnobotany. 5 [2 nd. ed.] . New Delhi .

2- Dwivedi , S.N. (2003). Ethnobotanical studies and conservational strategies of wild and natural resoures of Rewa district of madhy Pradesh, J. Econ . Tax . Bot., 27 (1) : 233-234.

3 -Sharma , N.K; Ahirwar , D., Jhade , D. and Gupta , S. (2009). Medicinal & pharmacological potential of *Nigella sativa* .A review . Ethnobotanial . Review . 13 : 946-55.

4 Dwivedi , S.N. , (1999) .Relivance of medicinal herbs used in traditional system of medicine .Farmavita.Net.

5 Khare ,C.P.(2004).Encyclopedia of Indian medicinal plants .Springes-Verlag Berlin Heidelberg, New York.

6- Bashandy,A.E.S.(2007).Effect of fixed oil of *Nigella sativa* on male fertility in normal and hyperlipidemic rats .Int.J.Pharmacol.3:27-33.

7- Ali,B.H.& Blunden, G.(2003).Pharmacological and toxicological properities of *Nigella sativa* .Phytother.Res.17:299-305.

8-Mohammad,M.A.;Mohamd,M.J.& Dradka,h.(2009).Effects of black seeds(*Negilla sativa*) on spermatogenesis and fertility of male albino rats .Med.Medical .Sci.4(2):386-390.

9- Shanshal ,S.(2004).Study of protective effect of black seed (*Nigella sativa* L.) on some testicular functions in Swiss mice exposed to Gamma-radiation .M.Sc.Thesis .Education college .Mosul University-Iraq.

10- Ali , A.B. & Ali , T. H . (2006) . Beneficial effects of *Nigella sativa* on the testis tissue of mice exposed to UV irradiation. J. Trace element in medicin & biology . 19:259-265.

11- Meral , I., Yener, Z.; Kahraman , T.& Mert , N. (2001) . Effect of *Nigella sativa* on glucose concentration , lipid peroxidation , antioxidant defense system and liver damage in experiment induced diabetic rabbits , J.Vet. Med. Physiol. Pathol. Clin. Med . Dec. 48(10):593-599.

12- El-Bahy,G.M.& Ismail ,Z.M.K.(1997).Effect of UV irradiation & *Negilla sativa* protective role on the mice liver tissues . Isotope & RAD.Res.29(1,2):39-52.

13- Prescott,L.F.& Critchely,J.A.J.H.(1983).The treatment of acetaminophen poisoning .Annu.Rev.Pharmacol.Toxicol.23:87-101.

14- Vale, J.A. & Meredith, T.J. & Goulding , R. (1981). Treatment of acetaminophen poisoning: the use of oral Methioine , Arch Inter . Med . 141:394-6.

15- Meredith ,T.J.;Prescott,L.F.& Vale, J.H.(1986).Editorial: why do patients still die from paracetamol poisioning ?Br.Med.J.293:345-6.

16- Hassan,W.B.;Kalifa,s.& Kitta, S.(1999).Effect 0f prolonged acetaminophen (panadol) ingestion on the mouse liver ,kidney and testis histology. Saudia J.Bio.6(2).168-178.

17- Balasubramanian , A.;Manimekalai, S.; Sing,A.& Ramakrishnan, S.(1980). Short and long term effect of aspirin on testes of albino rats . A histological & biochemical study . Ind.J. Exp.Biol .18:1408-1410.

18- Roomi , M.W.; Kalinovsky,T.; Ivanov, V.;Rath ,M.& Niedzwiecki,A.(2008) .A nutrient mixture prevents acetaminophen hepatic and renal toxicity in ICR mice. Human &Experimental Toxicology .27:223-230.

19- Bethesda,M.D. & Mc Evoy , G.K. (2007). American hospital formulary service.AHFS drug information -American society of health system pharmacists , 10 th. ed. .2182.

20- Goodman,P.B.& Gilman,A.G.(2001). The pharmacological basis of therapeutic ,10 th. ed. New York ,Mc Graw – Hill.704.

21- Anderson, B.J.; Holford ,N.H.G.;Woollard,G.A.& Chan,P.L.S.(1998). Paracetamol plasma & cerebrospinal fluid pharmakinetics in children .Br.J. Clin.Pharmacol.46:237-43.

22- Cramer,D.W.;Harlow,B.L.;TitusErnstoff,L.;Bohike,K.;Welch,W.R.& Greenerg,E.R.(1998) .Over-the-counter analgesic and risk of ovarian cancer,Lancet.351:104-7

23- Bjorkman,R.; Hallman,K.M.; Hedner,J.;Hedner,T.& Henning, M.(1994). Acetaminophen blocks spinal hyperalgesia induced by NMDA and substance P.Pain .57:259-64.

24- Ratnasooriya,W.D.;Darmasiri,M.G.&Wadsworth,R.M.(2000).Reduction in libido and fertility of male rats by administration of the nitric oxide (NO) synthase inhibitor N-nitro-L-arginine methyl ester .Int.J. Androl.23:187-91.reproductive competence of male rats .Asian .J.Androl.2:247-255.

25- Flower,R.J.& Vane, J.R.(1972) .Inhibition of prostaglandin synthatase in brain explains the antipyretic activity of acetaminophen .Nature. 240 : 410-1.

26- Ratnasooriya,W.D.& Lionel,N.D.W.(1983). Effect of polyphloretin phosphate (a prostaglandin receptor – blocking) drug on fertility of male rats. Ceylon . J. Sci. 16143-50.

27- Ratnasooriya,W.D& Jaykody,J.R.A.C.(2000).Long-term administration of large doses of paracetamol impairs the reproductive competence of male rats.Asian.J.Androl.2:247-255.

28- World Health Organization.(1999).WHO laboratory manual for the examination of the human semen and sperm- cervical mucus interaction. Cambridge University Press, Cambridge .

29- Persnell, J. K. & Schreebman, M. P. (1997). Humans Animals Tissue Techniques. 5th edn. The Jobus Hopkins press Ltd. London.

30- Scheffer, W.C. (1969). Statistic for biological sciences. 2nd edn. Addison Westey Publishing company . California, London, Amesterdam.

31- Halliwell ,B. ; Gutterridage , J.M. & Cross , C.E. (1992). Free radical untiioxidants & human disease . J, Lab, Clin , Med. 119: 598-6.

32- Mastsumoto , K. ; Okajo , A.; kobayashi, T.; Mitchell , J.B. ; krishna , M.C. & Endo, K. (2005). Estimation of free radical formation By ß-ray irradation in rat liver . J. Biochen . Biophys. Methos 63:79-90 .

33- Ke,Y.B. & Tso ,W.W. (1982) . Variations of Gossypol susceptibility in rat spermatozoa during spermatogenesis . Int. J. Fert.27(1):42-46.

34- Azzarito, C.; Boiardi,L.;Vergoni,W.;Zini,M.& Portilio,I. (1996). Testicular function in hypercholesteromic male patients during prolong simvastatine treatment .Horm.Metab.Res.28:193-198.

35- Chopra , R.N., Chopra , I.C. ; Handa, K.L. & Kapoor , L.D. (1958). Chopra Indigenous Drug of India , 2nd , ed., U.N. Dhar & Sous Pve. Ltd . Calcuta .

36- AL-Saaidi,J.A.A.; AL-Khuzai,A.L.D.& AL-Zubaydi,N.F.H. (2009).Effect of alcoholic extract of *Negilla sativa* on fertility in male rats.Iraqi Journal of Veterinary Sciences , 23(11):123-128.

37- AL-Saaidi,J.A.A.; AL-Khuzai,A.L.D.& AL-Zubaydi,N.F.H. (2009).Effect of alcoholic extract of *Negilla sativa* on fertility in male rats.Iraqi Journal of Veterinary Sciences , 23(11):123-128.

38- Rajesh,D.;Stenzel ,R.& Howard,S.(2003). Perillyl alcohol as a radio-/ chemo-sensitizer in malignant glioma.J.Biol.Chem.278:35968-78.