

**AMELIORATING AND PROTECTIVE ROLE OF QUERCETIN
AGAINST O-ANISIDINE TOXICITY ON SOME
REPRODUCTIVE ASPECTS OF LABORATORY MALE RATS
(*Rattus norvegicus*).**

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ABSTRACT

The present study assessed the effects of O-anisidine hydrochloride and the ameliorating effect of Quercetin dihydrate in laboratory rats. Sixteen male and thirty two female rats (*Rattus norvegicus*) were used and divided into eight equal groups of two male and four female rats each. The results revealed that the treatment with O-anisidine hydrochloride for 30 days (T1, T2 and T3 groups) caused significant decrease in the males body weights, sperm count, individual and massive sperm motility, testes weights, and epididymis weights as compared with control and (T4, T5, T6 and T7) groups at ($P \leq 0.05$). When Quercetin dihydrate was offered as an ameliorating agent, it showed a significant ameliorating effect by increasing the body weights, sperm count, individual and massive sperm motility, testes weights, and epididymis weights . When Quercetin dihydrate was offered alone in the ration of the sixth treated group (T6), it caused clear significant ameliorating effect on all sperm parameters comparing with all treated groups and the sperm count was even significantly higher than that of control group while the other aspects were similar to those of control group at ($P \leq 0.05$). Beside, O-anisidine caused significant decrease in the number of pregnant females, number of delivered litters, weight of litters and sex ratio, and it prevented the pregnancy from being occurred in the group where both male and females are treated with it (T1). When Quercetin was mixed with O-anisidine in the ration of (T7), it significantly ameliorated the pregnancy chances,

number of litters and the sex ratio as compared with the other groups but it didn't reach to significant level with control group at ($P \leq 0.05$).

INTRODUCTION

Aromatic amines is a well-known wide range of compounds family and Ortho-anisidine belongs to (1). The principal commercial use of O-anisidine is an intermediate in the manufacture of dyes and pharmaceuticals and as an intermediate in the manufacture of synthetic guaiacol and its derivatives (2). It has been known that O-anisidine is released from textiles and leather goods colored with azo dyes and a large part of the population may be exposed and as a constituent of cigarette smoke (3). Young children are exposed by oral suckling clothes which are colored with O-anisidine based dyes, and workplace exposure occurs by inhalation in the production and processing of O-anisidine, dermal contact during the formulation of O-anisidine based printing inks, general population in dermal contact with packing materials printed with O-anisidine based pigments; and man exposed indirectly via the environment (4).

A report made by (5), mentioned that O-anisidine metabolite (O-nitrophenol) causes testicular atrophy and inhibition of spermatogenesis in rats after oral exposure. Flavonoids are a group of naturally occurring polyphenolic compounds widely distributed as secondary metabolites in plant kingdom (6). Quercetin (3, 5, 7, 3, 4-pentahydroxy flavon), is one of the most prominent dietary antioxidants (7). Quercetin occurs in glycosylated form in French beans, broccoli, and apples and especially in onions (8). Quercetin is the most abundant antioxidant in the nature and has an antioxidant potential four times that of vitamin E (9). Quercetin has been reported to enhance the antioxidative defense system by up regulating antioxidant enzymes (10). It has many beneficial effects in human health, including cardiovascular protection, anticancer activity, anti-ulcer effects, anti-allergy activity, cataract prevention, antiviral activity and anti-inflammatory effects (11). Quercetin prevents oxidant injury and cell death by several mechanisms, such as scavenging oxygen radicals, protecting against lipid peroxidation and chelating metal ions (12). It was also reported that Quercetin ameliorates sperm aspect by preventing lipid peroxidation (13).

MATERIALS AND METHODS

Experimental animals and diets

Experimental animals

The experiment was conducted at the animal house of the Veterinary Medicine College–University of Basra, where 16 male and 32 female rats (*Rattus norvegicus*) of 170–175 grams weights were used. The experiment conditions were unified for all animals, where the room temperature was set between 20–25 C by the use of an air conditioner, and the daily light period was 12 hours by the use of two fluorescent lamps, and the humidity rate was about 50 %. Food and water were provided daily (*ad libitum*).

Experimental design

The animals were allocated into 8 groups; each group consists of 2 male and 4 female rats. The appropriate examinations were done after 30 days of the experiment. The groups were:

- ◆ Control group: Animals were maintained on a standard ration for 30 days.
- ◆ Treatment 1 (T1) group: In this group, both males and females were fed 1000mg/kg O-anisidine ration for 30 days.
- ◆ Treatment 2 (T2) group: In this group, the males only were fed 1000mg/kg O-anisidine hydrochloride ration and females on a standard ration for 30 days.
- ◆ Treatment 3 (T3) group: In this group, males were fed 1000mg/kg O-anisidine hydrochloride ration and females were fed 80mg/kg Quercetin dihydrate ration for 30 days.
- ◆ Treatment 4 (T4) group: In this group, males were fed 80mg/kg Quercetin dihydrate ration and females were fed 1000mg/kg O-anisidine hydrochloride ration for 30 days.
- ◆ Treatment 5 (T5) group: In this group, males were fed 80mg/kg Quercetin dihydrate ration and females were fed a standard ration for 30 days.
- ◆ Treatment 6 (T6) group: In this group, both males and females were fed 80mg/kg Quercetin dihydrate ration for 30 days.

◆ Treatment 7 (T7) group: In this group, both males and females were fed 80mg/kg Quercetin dihydrate + 1000mg/kg O-anisidine hydrochloride ration for 30 days.

Reproductive parameters

Once the female rats gave birth, the males are killed. Testes are cut and the epididymi are removed for the sperms viability measurements, and the rats litters are weighed by a sensitive balance at birth. The fertility and sex ratio are documented and the corpora lutea were counted. The parents of each group were mixed after the treatment period diminished, and they were left together for 16 days (14). Then the parents were separated again and females were left alone to deliver. The necessary tests such as massive and individual sperms movement, total sperms concentration, dead and alive sperms, and the sperms malformations were done after that.

A. Massive sperms motility measurement.

The massive sperm motility was done according to (15).

B. Individual sperms motility measurement.

The individual sperm motility was accomplished based on methods described by (16).

Statistical Analysis

In this study, ANOVA Analysis and LSD tests were used according to SPSS Statistics at the ($P \leq 0.05$) to find the means for all treatments (17).

RESULTS and DISCUSSION

The results showed that the treatment with O-anisidine hydrochloride for 30 days caused significant decrease in the sperm count, individual and massive sperm motility, testes and epididymi weights and it also caused a significant increase in the percentages of dead and abnormal sperms of the (T1, T2 and T3) groups as compared with the control and O-Anisidine+Quercetin treated groups (T7) at ($P \leq 0.05$) as it's clear from table (1). It's also clear from table (1) that when Quercetin dihydrate was offered as mixed with O-anisidine hydrochloride ration (T7) it caused a significant increase in sperm count, individual and massive sperm motility, testes and epididymi weights and it caused a significant decrease in the dead and abnormal sperm percentage as compared with those of (T1, T2 and T3) groups but these values were still significantly less than those of the control group at ($P \leq 0.05$). When Quercetin

dihydrate was used alone in the ration of (T4, T5 and T6) it caused a significant supportive effect on the sperm aspects of these groups as compared with (T1, T2, T3 and T7) groups and the improving effect was also higher than that of control group but not significantly at ($P \leq 0.05$).

The effect of O-anisidine on body weights of the male rats was elucidated in table (2), where; it caused a significant decrease in the body weights of the O-anisidine treated males from day 7 till day 28 of the experiment in (T1, T2 and T3) groups as compared with (T4, T5, T6 and T7) and the control groups at ($P \leq 0.05$). When Quercetin dihydrate was used as protective agent (T7) mixed with O-anisidine ration, it caused the body weight to elevate significantly as compared with (T1, T2 and T3) groups but it was still significantly less than that of the control group at ($P \leq 0.05$). Furthermore, when Quercetin dihydrate was used alone as an ameliorating agent (T4, T5 and T6) it caused the body weights to elevate significantly as compared with (T1, T2, T3 and T7) and it was even higher than the control group but without a significant difference at ($P \leq 0.05$).

Considering the fertility and fluency of the male and female rats, it is clear from table (3) that when both male and female rats were treated with O-anisidine hydrochloride alone (T1), the chances of pregnancy were nil. When either the males were treated with O-anisidine alone (T2 and T3) or when the females were treated alone (T4) the chances of pregnancy were one pregnant female from total 4 females per each group and there was a significant decrease in the number of delivered litters, number of delivered male litters, number of corpora lutea, weight of litters at birth, sex ratio and fertility percent as compared with (T5, T6 and T7) and the control groups at ($P \leq 0.05$). When both males and females were maintained on a mixed O-anisidine and Quercetin ration (T7), the chances of pregnancy and other aspects increased significantly as compared with the other groups but they were significantly less than (T5 and T6) and control groups at ($P \leq 0.05$). Beside, when both males and females were maintained on Quercetin dihydrate alone ration (T6) or the males only were maintained Quercetin and females were maintained on a standard rations, all the fertility aspects elevated significantly more than all other groups and even were higher than that of the control group but not significantly at ($P \leq 0.05$).

All the mentioned above effects of O-anisidine can be explained due to its very toxic metabolites, where ring oxidation, N-glucuronidation, N-acetylation, and N-oxidation are the major metabolic pathways of arylamines in mammals (18).

One of the most effects of these metabolites is the formation of reactive oxygen species (ROS) especially by the metabolite O-aminophenol and nitrobenzene like superoxide radicals or hydrogen peroxide. ROS can induce oxidative damage to the cell and can form a very stable structure by extracting electrons from other sources (19). ROS are also able to generate other forms of ROS. Superoxide can be dismutated into H₂O₂ and oxygen. H₂O₂ has the ability to form the more damaging $\cdot\text{OH}$, through a combination of the Fenton and Haber-Weiss reactions (20). The ROS which are not neutralized, can target biological molecules such as DNA, lipids, proteins, and carbohydrates, which can result in cell dysfunction or cell death. Hydroxyl radical is an extremely reactive free radical that can react with various biomolecules such as membrane lipids. The mechanism of action of Quercetin has been attributed largely to the antioxidant properties, which are known to augment GSH and antioxidant enzyme levels and scavenge lipid peroxides. A concept is now emerging of “adaptogenic drugs” - drugs that increases non-specific resistance to variety of stresses (21).

Table (1). Role of Quercetin dihydrate against O-anisidine hydrochloride on sperm viability aspects of rats. Different letters refer to significant differences among groups at (P≤0.05).

PARAMETERS GROUPS	Sperm count (nx106/mm3)	Massive motility (%)	Individual motility (%)	Dead Sperm (%)	Abnormal Sperm (%)	Testes wt (mg)	Epididymis Wt (mg)
Control	b 195.2 ±0.3	a 90 ±0.0	a 90 ±0.0	b 11 ±1.4	b 12.5 ±2.1	b 0.79 ±0.01	a 0.29 ±0.01
Anisidine	c 66.5 ±1.0	b 10 ±0.0	b 10 ±0.0	a 96 ±1.4	a 31 ±1.4	c 0.43 ±0.02	b 0.10 ±0.0
Anisidine +Quercetin	d 125.7 ±1.5	c 77.5 ±3.5	c 47.5 ±3.5	c 32.5 ±3.5	c 20 ±1.4	d 0.64 ±0.04	c 0.26 ±0.01
Quercetin	a 198.1 ±0.7	a 90 ±0.0	a 90 ±0.0	b 9 ±1.4	b 9 ±1.4	a 0.86 ±0.05	a 0.29 ±0.0
LSD	2.9	12.5	37.5	21.5	7.5	0.15	0.03

Table (2). Role of Quercetin dihydrate against O-anisidine hydrochloride on body weight of rats. Different letters refer to significant differences among groups at ($P \leq 0.05$).

PARAMETERS GROUPS	Initial weight (gm)	Day 7 weight (gm)	Day 14 weight (gm)	Day 21 weight (gm)	Day 28 weight (gm)
Control	a 177.06 ±1.29	a 183.26 ±1.28	a 188.46 ±1.29	a 195.66 ±1.27	a 201.86 ±1.29
Anisidine	a 176.90 ±1.45	b 179 ±1.46	c 180.08 ±1.58	c 181.08 ±1.56	c 181.18 ±1.58
Anisidine+ Quercetin	a 177.18 ±1.70	a 182.28 ±1.69	b 187.28 ±1.70	b 192.18 ±1.68	b 197.18 ±1.66
Quercetin	a 176.8 ±1.12	a 184.5 ±0.96	a 190.3 ±0.94	a 197.2 ±0.87	a 204.9 ±1.11
LSD	0.00	2.3	2.17	1.6	3.1

Table (3). Role of Quercetin dihydrate against O-anisidine hydrochloride on fertility aspects of rats. Different letters refer to significant differences among groups at (P<0.05). **Note: A.m. = Anisidine treated males; A.f. = Anisidine treated females; Q.m. = Quercetin treated males; Q.f. = Quercetin treated females; and n.f. = normal females.

Aspects **Treatments	No. of Pregnant females	No. of litters	Birth weight of litters	No. of corpora lutea	No. of male litters	No. of female litters	Sex ratio %	Fertility percent %
Control	a 4 ± 0.0	b 36 ± 1.8	a 4.82 ± 1.0	a 39 ± 0.8	c 20 ± 1.2	b 16 ± 0.9	a 55.5 ± 0.9	a 100 ± 0.0
A.m.+A.f.	d 0 ± 0.0	d,e 0 ± 0.0	b 0 ± 0.0	d 0 ± 0.0	f,e 0 ± 0.0	e 0 ± 0.0	b 0 ± 0.0	d 0 ± 0.0
A.m+n.f.	c 1 ± 0.5	d 4 ± 2.0	a 4 ± 2.0	b 27 ± 13.5	e 1 ± 0.5	d 3 ± 1.5	b,a 25 ± 12.5	c 25 ± 12.5
A.m+Q.f.	c 1 ± 0.5	d 5 ± 2.5	a 4 ± 2.0	b,a 30 ± 15.0	e 2 ± 1.0	d 3 ± 1.5	a 40 ± 20.0	c 25 ± 12.5
Q.m+A.f	c 1 ± 0.5	d 2 ± 1.0	a 3.5 ± 2.0	c,b 15 ± 7.5	e 1 ± 0.5	e 1 ± 0.5	a 50 ± 25.0	c 25 ± 12.5
Q.m+n.f.	a 4 ± 0.0	b 37 ± 1.7	a 4.75 ± 1.4	a 41 ± 1.2	b 22 ± 0.8	b 15 ± 0.8	a 59.4 ± 0.5	a 100 ± 0.0
Q.m+Q.f	a 4 ± 0.0	a 42 ± 1.3	a 4.9 ± 1.7	a 46 ± 0.9	a 24 ± 1.2	a 18 ± 1.2	a 57.1 ± 0.8	a 100 ± 0.0
(A+Q M)+(A+Q F)	b 3 ± 0.5	c 23 ± 3.9	a 4.26 ± 0.8	b,a 32 ± 1.2	d 10 ± 0.8	c 13 ± 1.2	a 43.4 ± 0.5	b 75 ± 0.0
LSD	0.75	3.2	3.0	13.7	1.5	1.7	30.7	25.0

الدور المحسن و الوقائي للكويرستين ضد التأثير السمي للأنيسدين في بعض معايير التكاثر للجرذان المختبرية (*Rattus Norvegicus*)

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أخلاصة

تناولت هذه الدراسة تقييماً لتأثير الأورثو أنيسدين هيدروكلورايد والتأثير المحسن للكويرستين داي هايدريت في الجرذان المختبرية. لقد تم استخدام ١٦ ذكراً و ٣٢ أنثى من الجرذان المختبرية والتي تم توزيعها في ٨ مجاميع متساوية بواقع ٢ ذكور و ٤ إناث لكل مجموعة. تم فصل الذكور عن الإناث طيلة ال ٣٠ يوماً من التجربة ومن ثم أدخلت إلى الإناث المجموعة الأولى كانت مجموعة السيطرة والتي تم تغذية الحيوانات فيها على عليقة قياسية لمدة ٣٠ يوماً. المجموعة الثانية كانت مجموعة المعاملة الأولى (T1) وفيها كل من الذكور والإناث تم تغذيتها على عليقة قياسية تحتوي ٠.٠٠٠ ملغم/كغم أورثو أنيسدين هيدروكلورايد. مجموعة المعاملة الثانية (T2) وفيها فقط الذكور غذيت على عليقة الأنيسدين والإناث على

عليقة قياسية. مجموعة المعاملة الثالثة (T3) تم تغذية الذكور على عليقة الانيسيدين والاناث على عليقة تحتوي ٨٠ ملغم/كغم كويرستين داي هيدريت. مجموعة المعاملة الرابعة (T4) تم تغذية الذكور على عليقة كويرستين والاناث على عليقة الانيسيدين. مجموعة المعاملة الخامسة (T5) تم تغذية الذكور على عليقة الكويرستين والاناث عليقة قياسية. مجموعة المعاملة السادسة (T6) تم تغذية كل من الذكور والاناث على عليقة الكويرستين. مجموعة المعاملة السابعة (T7) كل من الذكور والاناث تم تغذيتها على عليقة تحتوي الانيسيدين ١٠٠٠ ملغم/كغم والكويرستين ٨٠ ملغم/كغم.

اظهرت النتائج ان معاملة الحيوانات بالانيسيدين لمدة ٣٠ يوما (T1, T2 and T3) قد تسبب بنقصان معنوي ($P \leq 0.05$) في اوزان جسم الذكور، عدد النطف، الحركة الفردية والجماعية للنطف، اوزان الخصى واوزان البرابخ مقارنة مع مجموعة السيطرة والمجاميع (T4, T5, T6 and T7). عندما استخدم الكويرستين كعامل محسن فانه تسبب بتاثير محسن معنوي تمثل بزيادة اوزان الجسم، عدد النطف، الحركة الفردية والجماعية للنطف، اوزان الخصى واوزان البرابخ. عندما استخدم الكويرستين لوحده في عليقة مجموعة المعاملة السادسة فانه سبب تاثيرا محسن معنويا في كل معايير النطف مقارنة مع جميع مجاميع المعاملة وكان عدد النطف كان ايضا اكبر معنويا حتى من مجموعة السيطرة بينما كانت المظاهر الاخرى مساوية لمجموعة النطف معنويا عند مستوى احتمال ($P \leq 0.05$). بالاضافة الى ذلك تسبب الانيسيدين بانخفاض معنوي في عدد الاناث الحوامل، عدد المواليد، اوزان المواليد والنسب الجنسية وقد منع حدوث الحمل في مجموعة المعاملة الاولى حيث الذكور والاناث معاملة بالانيسيدين. عندما مزج الكويرستين مع الانيسيدين في عليقة المجموعة السابعة فانه حسن معنويا فرص حدوث الحمل، عدد المواليد ونسبة الجنس مقارنة بالمجاميع الاخرى لكن دون الوصول لقيم مجموعة السيطرة عند مستوى احتمال ($P \leq 0.05$).

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