

Effect of Royal Jelly on Sperm Parameters in Aluminum-Treated Male Rats

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Abstract:

Background: Aluminum (Al) metal is abundantly present in the earth's crust. Aluminum is present in many manufactured foods and medicines and is also added to drinking water during purification purposes, and this has allowed its easy access into the body. Aluminum has been proposed as an environmental factor that may contribute to some diseases. Different forms of aluminum are environmental xenobiotics that induce free radical-mediated cytotoxicity and reproductive toxicity. Royal jelly is a substance produced by worker honey bees. If fed to an ordinary female bee in the larval stage, royal jelly will transform her into the queen bee. Scientific research on royal jelly has revealed that it benefits sexual performance.

Methods and Materials: Fifty adult male albino rats were used in this study. They were randomly divided into five groups. The first group served as control group (negative group). The second group was received 20 mg AlCl₃ /kg body weight (positive group). The other groups were received the same dose of AlCl₃ and subdivided into three groups according to the concentration of royal jelly (RJ). Three concentrations of RJ were investigated (50, 100 and 200 mg/kg). All treated doses were given orally by gastric intubation and the experiment was continued daily for 60 days. This study included the studying the sperm parameters in tail of epididymis, measuring the values of some hormones including testosterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH) and estimate the level of malondialdehyde (MDA) in blood serum.

Results: The supplementation of aluminum-treated animals with the royal jelly resulted in an appreciable improvement in all the studied reproductive parameters, hormonal level and MDA. Moreover, increasing degree of amelioration was in correlation with the amount of progressive concentrations of royal jelly.

Conclusion: This study showed that RJ has alleviating effect and protective role in the condition of aluminium -induced oxidative stress.

Key words: Royal jelly, aluminum, sperm parameters, rats.

Introduction

Aluminum (Al) is available and widely distributed in nature. It is found in soil, rocks, water, food and air. The aluminum is used in various industries such as household utensils, foils, in aircrafts and vehicles industry, construction of buildings as well as in the military industry (1). Moreover, aluminum compounds are used in the water purification, involved in drugs and food industry or in various other purposes, such as consumer products including

antacids, buffered aspirin, astringents, food additives, antiperspirants and cosmetics (2).

Generally, most of humans are in direct and inevitable exposure for most metals within their environment due to their ubiquity in nature, contaminated air, water, soil and food and wide use in the industry (3). So, aluminum is one of these elements that are exposed to the daily lives of humans. There is an increasing concern over the aluminum toxicity in animals and human. Reproductive toxicity in human

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and animal can be initiated after being exposed to aluminum compounds. The male reproductive toxicity has been reviewed in several studies (4, 5, 6).

The honeybee workers are the source of royal jelly (RJ) production as they are excreted from mandibular and hypopharyngeal glands, and this food is essential for the maintaining and developing of the queen bees (7). Many studies have dealt with the functional significance of RJ because of its importance to human health. In one study, the results showed that hematological and immunological parameters increased in mice treated by bee RJ (8). On the other hand, The antioxidative activities of commercial honey, royal jelly, and propolis have been studied by Nagaia *et al.* (9). The present study aims to investigate the toxic effects of aluminum chloride ($AlCl_3$) in the reproductive efficiency of albino male rats and protective role of the RJ towards the aluminum toxicity.

Materials and methods

Experimental animals

Adult albino male rats weighing between 215-288 gm were used in this study. They were housed in plastic cages at Faculty of Veterinary Medicine/ University of Kufa/ Iraq, and kept in controlled environment. Commercial food and tap water were provided to animals *ad libitum*.

Experimental design

Fifty sexually mature male rats were randomly distributed into five groups (10 rats each). All experimental rats, except normal control animals (negative group), were given orally by gavage daily with aluminum chloride ($AlCl_3 \cdot 6H_2O$, Fluka Chemicals -Switzerland) at concentration 20 mg/kg body weight (10).

The treated animals were subdivided into four groups. The positive control group was not treated with RJ, while the animals of the remaining three groups were given different concentrations of RJ (50, 100 and 200 mg/kg/day) respectively. The orally dosages of RJ were determined according to Galaly *et al.* (11). The concentrations of $AlCl_3$ and RJ that dissolved in distilled water depended on body weight. The period of exposure with $AlCl_3$ and treatment with RJ was continued for 60 days (12).

Sample collection

At the end of the experimental period, all rats were anesthetized, using a mixture of ketamine and xylazine i.m., and then they were sacrificed (13). The blood sample was obtained from animal through heart puncture by using a 5 ml disposable syringe for biochemical tests. The blood was placed in tubes without anticoagulant and centrifuged at 3000 rpm for 10 minutes. The blood serum was separated and kept at in refrigerator at $-20^\circ C$ until the time of analysis (14). For male fertility parameters, epididymis were removed, cleaned from connective tissue. Thereafter, the epididymis was put in normal saline and taken for analysis.

Measurement of sperm parameters in epididymis

The sperm concentration in epididymal tail was counted according to method of Seed *et al.* (15), using the light optical microscope. The tail of epididymis was cut into small pieces using surgical blades and the content squeezed gently in clean watch glass. The content was homogenized with 9.8 ml neutral formalin solution to ensure the release of the sperms for epididymal sperm count. Calculation of sperms was performed by using hemocytometer slide at 400X magnification.

The percentage of live and morphologically abnormal sperms were measured in the glass slide smear prepared from the suspension of epididymal tail content by using eosin-nigrosin stain (16) diluted with 3% sodium citrate. For each sample, at least 10 microscopic fields and 500 sperms from different fields were observed at 400X magnification under light microscope, in order to calculate the percentage of viability and abnormality of sperms (17).

In this technique, the head of live sperm was not stained with pigment, while the head of dead sperm cell seemed to be pink (18). While, the morphological abnormal sperms include hook, larger head, curly head, double head and tail or without head and tail (19).

Determination of FSH, LH and testosterone levels

The levels of serum hormones, namely, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone were evaluated, using enzyme linked immune sorbent assay (ELISA) method, according to the procedures provided by the manufacturer's instructions (Accu-Bind, USA).

Measurement of malondialdehyde (MDA) level

Malondialdehyde (MDA) activity was evaluated according to the technique of Gutteridge (20). This method is based on the color that formed by chemical reaction between MDA and thiobarbituric acid (TBA) which is measured at 532 nm using a spectrophotometer.

Statistical analysis

Statistical package for social sciences (SPSS) program were used to analyze the data of present study. Analysis for statistical differences of means between the animal groups was done using one-way analysis of variance (ANOVA). The least significant difference (LSD) test was performed to determine the significant variances. $P \leq 0.05$ was considered to be significant (21).

Result and discussion

Effect of $AlCl_3$ and RJ on sperm parameters

Sperm concentration

The results obtained from the present study show a significant decrease ($P < 0.05$) in sperm concentration of epididymis in $AlCl_3$ - treated group in compare to normal control group. On the other hand, treatment with RJ at concentration 200 mg/kg in combination with $AlCl_3$ showed a significant increase ($P < 0.05$) in sperm count in comparing with $AlCl_3$ group, while no significant difference were observed in the rats that received 50 and 100 mg/kg of RJ (Table 1).

Sperm count is one of the most sensitive tests for spermatogenesis and fertility evaluation (22). In rats, $AlCl_3$ has marked effects on the testis and epididymis, causing arrest of spermatogenesis (23).

Table 1: Effect of $AlCl_3$ and RJ on sperm concentration, sperm viability and sperm abnormality after 60 days.

Groups	sperm concentration ($10^6/ml$)	sperm viability (%)	sperm abnormality (%)
C	159.56 $\pm$ 5.77	93.3 $\pm$ 1.20	4.6 $\pm$ 0.16
$AlCl_3$	90.20 $\pm$ 8.83 ^a	15.9 $\pm$ 0.87 ^a	29.0 $\pm$ 1.04 ^a
$AlCl_3$ +RJ50	90.36 $\pm$ 6.79 ^a	29.7 $\pm$ 0.92 ^{a,b}	9.0 $\pm$ 0.57 ^{a,b}
$AlCl_3$ +RJ100	107.69 $\pm$ 6.31 ^a	50.3 $\pm$ 2.89 ^{a,b,c}	8.0 $\pm$ 2.28 ^{a,b}
$AlCl_3$ +RJ200	146.39 $\pm$ 11.54 ^{b,c,d}	84.9 $\pm$.98 ^{a,b,c,d}	6.1 $\pm$ 0.72 ^{b,c}

- C: control group, $AlCl_3$: aluminum chloride treated group, $AlCl_3$ +RJ: aluminum chloride and royal jelly-treated groups.

- a: Significantly different from control group; b: Significantly different from $AlCl_3$ group ; c: Significantly different from $AlCl_3$ +RJ 50 group; d:Significantly different from $AlCl_3$ + RJ 100 group.

- Data are expressed as mean $\pm$ standard error.

- One-way ANOVA with LSD test at $P < 0.05$.

Hidaka *et al.* (24) suggested that presence of steroidal hormones within the RJ might be considered one reason for enhancement in the sperm count because the central role of testosterone in the spermatogenesis. Furthermore, the zinc is another important ingredient of RJ, which leads to series of enormous benefits on the body and reproductive system can include an increase in the number and mobility of sperm cells and regulation of prostate function (25). In another study, Robak *et al.* (26) observed a positive relationship between sperm density and zinc level in men.

Sperm viability

Table (1) shows the different effects of $AlCl_3$ and RJ on sperm viability in epididymis. The result of this study exhibited a marked decrease ($p < 0.05$) in percentage of sperm viability in the male rats of $AlCl_3$ -treated group. While the administration of RJ at concentrations 50, 100 and 200 mg/kg resulted in significantly increases ($p < 0.05$) in the percentage of sperm viability in comparison to $AlCl_3$ - treated group. Dawson *et al.* (27) stated that the decline in percentage of live sperm and motility in human due to elevation of aluminum concentrations in spermatozoa and seminal plasma. Moreover, Zatta *et al.* (28) demonstrated that activity of some important mitochondrial enzymes (e.g. aconitase), which is considered necessary during Krebs cycle, and can be influenced in the presence of aluminum. Hence,

mitochondrial dysfunction may lead to disturbance in sperm viability and motility (29).

The present study indicated that oral administration of RJ in combination with aluminum minimized its hazards in regard to sperm viability. This result agrees with numerous studies which have demonstrated that RJ with its antioxidant properties has positive effects for increase the activity of epididymis and then enhancement sperm viability and general reproductive status (30; 31; 32; 33; 18 and 34).

Sperm abnormality

The effects of $AlCl_3$ and RJ on sperm abnormality in rats are shown in Table (1). The results indicate a significant increase ($p < 0.05$) in percentage of sperm abnormality of $AlCl_3$ -treated group in comparing with control group, whereas treatment male rats with RJ showed significant decreases ($p < 0.05$) in sperm abnormality in compare with $AlCl_3$ -treated group.

The increase in the sperm abnormality may be due to the elevation of the oxidative stress which induced by $AlCl_3$. Moreover, the relationship between the oxidative stress induced by xenobiotics and sperm abnormality has been reported (35). According to Abdul-Rasoul *et al.* (36), the elevation in oxidative stress or insufficiency of androgen hormones is considered an important factor for the increase of the sperm abnormality of male rats induced by $AlCl_3$, in turn, these factors lead to reduction in sperm maturation and secretory functions of epididymal cells.

Generally the sperm cells produce controlled concentrations of ROS needed for fertilization. However, when the free radicals were produced in high concentrations can directly damage the sperm cells (37). In fact, Phospholipids and Polyunsaturated fatty acids consider the main constituents of sperm cell membrane and these compounds vulnerable to oxidative damage induced by free radicals (38). It is well known that the free radicals can induce DNA damage in meiotic chromosomes (39) and also interfere with the differentiation process during the spermatogenesis (40). In parallel, the increase in sperm deformity of male rats after being treated with $AlCl_3$ may be attributed to the increase in thiobarbituric acid reactive substances (TBARS), because any increase in TBARS level can lead to harmful effect on midpiece of sperm (41).

This study revealed a marked decrease in the abnormal sperm percentage when the orally RJ was introduced in combination with $AlCl_3$ for male albino rats, and this may be due to the antioxidant influence of RJ against the adverse effect of $AlCl_3$ on spermatogenic cells. Thus, our result is in agreement with previous study (31) who found that RJ has a beneficial role for reduced the abnormal sperm in male adult rats which treated with hydrogen peroxide.

Graham (42) reported that the presence of different amino acids and vitamins in component of RJ reduce or prevent some abnormality to occur in sperm shape. Furthermore, Inoue *et al.* (43) observed that RJ reduced tissue DNA oxidative damage and increased life span. Cavusoglu *et al.* (44) found that genotoxicity and oxidative damages induced by cadmium toxicity can be prevented in albino mice by treatment with RJ and this recovery can be attributed to the role of RJ against the chromosomal aberrations.

Effect of $AlCl_3$ and RJ on some hormonal levels LH and FSH

The results showed in Table (2) indicate a significant decrease ($P < 0.05$) in both LH and FSH levels in $AlCl_3$ -treated group in comparing with control group.

The data of this study showed that interference of RJ with $AlCl_3$ at concentrations 100 and 200 mg/kg of body weight showed significant increases ($P < 0.05$) in LH and FSH levels compared to $AlCl_3$ -treated group, while no significant different was recorded in rats that received 50 mg/kg of RJ.

The decline of LH and FSH levels in blood serum may be attributed to accumulation of aluminum in the pituitary gland and testis which may be lead to function disturbance in the pituitary-testicular axis in rats (45). Moreover, the decrease in LH and FSH levels reflect the decline in the level of gonadotropin releasing hormone (GnRH) which secreted from hypothalamus and play an important role in regulating LH and FSH secretion from pituitary gland (46).

Similar observations were noted in male rats by Shahraki *et al.* (47) and Ige and Akhigbe (12) who recorded a decrease in the levels of LH and FSH. However, contradictory result has been reported by Mayyas *et al.* (48) in male mice.

On the other hand, RJ contains about 1 mg / g dry weight of acetylcholine within its contents (49). It is interesting that this neurotransmitters stimulates the secretion of GnRH from the hypothalamus (50).

Consequently, the using of RJ causes increasing in secretion of GnRH from hypothalamus, in turn, this leads to increase in the releasing of LH and FSH to blood serum.

Table 2 : Effect of AlCl₃ and RJ on LH ,FSH and testosterone hormone after 60 days.

Groups	LH levels (mIU/ml)	FSH levels (mIU/ml)	Testosterone levels (ng/ml)
C	2.96±0.26	1.79±0.07	4.59±0.11
AlCl ₃	0.70±0.05 ^a	0.59±0.04 ^a	1.86±0.13 ^a
AlCl ₃ +RJ50	0.70±0.05 ^a	0.72±0.00 ^a	2.33±0.17 ^{a,b}
AlCl ₃ +RJ100	1.50±0.05 ^{a,b,c}	0.81±0.04 ^{a,b}	3.35±0.14 ^{a,b,c}
AlCl ₃ +RJ200	2.53±0.29 ^{b,c,d}	1.16±0.08 ^{a,b,c,d}	4.23±0.13 ^{b,c,d}

-C: control group, AlCl₃: aluminum chloride treated group, AlCl₃+RJ: aluminum chloride and royal jelly-treated groups.
 - a: Significantly different from control group; b: Significantly different from AlCl₃ group ; c: Significantly different from AlCl₃+RJ 50 group; d: Significantly different from AlCl₃+ RJ 100 group.
 - Data are expressed as mean ± standard error.
 - One-way ANOVA with LSD test at P<0.05.

Testosterone hormone

The effect of AlCl₃ and RJ on testosterone levels are shown in Table (2). The results revealed a significant decrease (p<0.05) in AlCl₃-treated group in comparison with control group. Treated rats with RJ at concentrations 50, 100 and 200 mg/kg of body weight showed significant increases (p<0.05) in comparison with AlCl₃-treated group.

As it is known, testosterone hormone is released from the interstitial cells (cells of Leydig) by stimulation of LH (51). So, the reduction in LH level in this study induced by AlCl₃ exposure resulting in decline of serum testosterone concentration. It has been reported that aluminum exerted a significant adverse effect on the steroidogenesis as well as increased production of nitric oxide, induced by excessive aluminum, and might inhibit testosterone levels (52). The result of our study coincides with the findings of Hali *et al.* (10) and Moselhy *et al.* (53) who recorded a marked decrease in testosterone in serum of male rats.

Generally, aluminum accumulation and low concentration of testosterone will eventually reflected on the sex organ functions and may result in severe reduction in male fertility and libido (54).

In other hand, results of this study showed that the testosterone hormone was dramatically increased with the increase of the RJ dose which administrated to the male rats. Our data demonstrated that RJ was in all concentrations resulted in notable increase in testosterone level when compared to AlCl₃ group. Thus, our results are in line with data reported by others. Recently Najafi *et al.* (55) recorded that RJ caused a significant increase in level of testosterone in serum of male mice induced by oxymetholone.

The enhancement in the level of testosterone may be attributed to the role of RJ in increasing the concentration of LH, which is considered the most important factor for stimulation secretion of testosterone from interstitial cells (56). Furthermore, Al-Sanafi *et al.* (57) demonstrated that testosterone could be increased as a result of exogenous supplementation through RJ. Furthermore the RJ contains this hormone in amount 0.20 mg/100 fresh weight (58). In addition, according to Hunt *et al.* (59), the zinc found in RJ is responsible for the elevation of testosterone level in the serum of young men.

Effect of AlCl₃ and RJ on MDA level

Effect of AlCl₃ and RJ on malondialdehyde (MDA) levels are presented in Table (3). Aluminum administration resulted in a significant increase (p<0.05) in the MDA level of control group. Treatment of male rats with combination of RJ and AlCl₃ showed significant decreases (p<0.05) in MDA levels in comparison with AlCl₃-treated group.

Under normal cellular conditions ROS and free radicals can be generated. Nevertheless, these compounds are immediately removed by major scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX). Therefore, ROS can serve as key signal molecules in both physiological and pathological processes involving reproductive fecundity (12).

If any imbalance occurs between free radical species and body's scavenging ability, the oxidative stress can be initiated (60). One of the most important biological outcomes of oxidative cellular damage is the lipid peroxidation (61). This important consequence of oxidative injury has been found to play an essential role in several carcinogenesis and toxicological process (62).

In general, the oxidative stress can exert their particular effect on the testis tissues (63). The

peroxidation of polyunsaturated fatty acids in the plasma membrane leads to disturbances in sperm functions (64). Also, Storey (65) stated that defective which occurs in sperm function may be attributed to increased lipid peroxidation and impaired antioxidant defense system.

Table 3: Effect of AlCl₃ and RJ on MDA after 60 days.

Groups	MDA levels (μmol/ml)
C	12.33±1.20
AlCl ₃	42.33±1.45 ^a
AlCl ₃ +RJ50	31±1.00 ^{a,b}
AlCl ₃ +RJ100	27±1.00 ^{a,b,c}
AlCl ₃ +RJ200	17.33±0.33 ^{a,b,c,d}

- C: control group, AlCl₃: aluminum chloride treated group, AlCl₃+RJ: aluminum chloride and royal jelly-treated groups.

- a: Significantly different from control group; b: Significantly different from AlCl₃ group; c: Significantly different from AlCl₃+RJ 50 group; d: Significantly different from AlCl₃+RJ 100 group.

- Data are expressed as mean ± standard error.

- One-way ANOVA with LSD test at P<0.05.

In experimental studies, the most common biomarker used to investigate lipid peroxidation in sperm is MDA, which is measured by thiobarbituric acid test (TBA) (66). The reason is that MDA is the most important products are produced during the process of lipid peroxidation (64).

In current study, the recorded high concentrations of MDA in AlCl₃-treated group may be associated with fact that exposure to high amount of aluminum leads to increase in ROS and decreased antioxidant enzyme activities in serum and testis tissue. Thus, it was suggested that lipid peroxidation may be a potential cause of increased MDA level (54).

RJ is a dietary natural antioxidant which referred to it for carry out the regulation of a number of important physiological and pathological processes (67). Therefore, RJ was used in this study to provide antioxidant protection against AlCl₃ toxicity. Typically, RJ caused a perceptible reduction in MDA serum level towards the normal values of control group. This decline may be attributed to the biological and antioxidant effects of the active ingredients of RJ. Bărnuțiu *et al.* (68) referred in their review that RJ contains collection of vitamins; among them vitamin E and vitamin C. As it's known, vitamin C can act as antioxidant, both by itself or by interacting with

vitamin E, for protecting the cell membrane against oxidative stress induced by several factors (69).

Additionally, it have been reported possible mechanisms for antioxidant activity of RJ include the following: (1) Three tyrosyl dipeptides (Lys-Tyr, Arg-Tyr, and Tyr-Tyr) of RJ have high antioxidant activity which scavenge the free radicals by hydroxyl group of their hydrogen atoms (70), (2) RJ regulates the levels of certain enzymes in response to lipid peroxidation such as cytochrome P450, 4A14 enzymes and glutathione-s-transferase. This regulation causes reduction in peroxidation of endogenous lipids (71).

Conflict of interest

None

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