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Protective Role of Royal Jelly against Histological Effects of Aluminum Chloride on Testes of Male Rats

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Abstract:
The aim of this study is to investigate the protective role of the royal jelly on the histological alterations of testes induced by aluminum chloride ($AlCl_3$). The first group of the rats was negative control group. The four treatment groups were received 20 mg $AlCl_3$ /kg body weight, one of them was considered the positive control group, The three other groups were received the same dose of $AlCl_3$ and subdivided according to the different concentration of the concentrations of royal jelly (50, 100 and 200 mg/kg). All treated doses were given orally by gastric intubation and the experiment was continued daily for 60 days. Due to experimental intoxication with $AlCl_3$, the microscopic examination for the testes in rats revealed numerous histological lesions in the seminiferous tubules and the interstitial tissue. In contrast, the histopathologic changes of testes were partially reversed by treatment with royal jelly and the testes appeared with nearly normal structure. It may be concluded that royal jelly revealed protective effect against the reproductive toxicity of $AlCl_3$.

Key words: royal jelly, aluminum, testes, seminiferous tubules, rats.

Introduction:

Aluminum is the most abundant metal and the third most abundant element, after oxygen and silicon, in the earth's crust (1). Aluminum is one of the elements that enter widely in large quantities in daily life; so many sources contain aluminum within their contents as food additives, toothpastes and deodorants (2). Besides, aluminium compounds have several medical applications such as vaccine, antacids, antiperspirant, buffered aspirins, phosphate binders and allergen injections (3). It has been observed that the accumulation of this element to the extent of toxic concentrations causes a variety of environmental damages in terrestrial and aquatic systems, so numerous studies have been dedicated to evaluate the toxic effects of aluminum in both human and animal, especially the potential influences of this element in the reproductive system. Moreover, the effect of aluminum on male and female fertility has become an area of growing concern (1).

Reproductive toxicity in human and animal can be initiated after being exposed to aluminum compounds. Testicular tissue damage has been associated with ingestion and accumulation of aluminum in target organs (4,5). Aluminum is able to interfere with several biological functions in regard to the reproductive toxicity including increased oxidative stress, alterations in membrane function, disruption in cell signaling, altering or inhibiting the enzyme activities and impairment of blood testis barrier (6).

On the other hand, the royal jelly (RJ) is one of the products of honeybee workers that secreted by the salivary glands and is devoted mainly as food for the queen throughout the larval period, while other nurse honeybee larvae are fed royal jelly for only three days (7). RJ can be exerting their positive effects on human body due to its natural pharmacological activities. It is usually used in folk and official therapy because it

is consider as beneficial and effectual remedy. However, it is regarded as a controversial dietary supplement (8). The pharmacological activities of RJ include the following: antioxidative activity, insulin-like action, hypotensive and blood regulatory actions, anti-inflammatory action (antibacterial, antiviral and fungicidal effects), wound healing effect, antihypercholesterolemic activity, hepatoprotective effect, immunomodulating effect, anti-allergic effect, antitumor action, tonic action, neurotrophic action and anti-aging effect. These important activities have been reviewed in some studies (7,8,9,10). Recent studies have shown that the RJ has important functional effects, especially in the field of reproduction and fertility. So, because of its unique qualities and desired characteristics has been used widely in medical treatments, health foods and in cosmetics (7).

The present study aims to investigate the histological toxic effects of aluminum chloride ($AlCl_3$) in the testis of albino male rats and protective role of the RJ towards the aluminum toxicity.

Materials and Methods:

Royal jelly sample

The Royall jelly (RJ) sample was obtained from a beekeeper shop in Najaf province (a region 160 km south Baghdad) during the year 2014. RJ sample were kept in cold condition and until its processing.

Experimental animals

Healthy adult albino Sprague-Dawley rats weighing between 215-288 gm were used in this experiment. They were housed in separated plastic cages at Faculty of Veterinary Medicine/ University of Kufa/ Iraq, and kept in controlled environment of 22-25 °C. Commercial food (pellets) and tap water were provided to animals *ad libitum.* Rats were left to acclimatize for at least two weeks before the start of the experiment. None of the rats had any clinically evident infections.

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 ($AlCl_3$; $6H_2O$. Fluka Chemicals - Switzerland) at concentration 20 mg/kg body weight. This dose was used according to the previous study of Hala *et al*. (2010) (11).

The treated animals were subdivided into four groups. The positive control group did not treat with RJ, while the animals of the remaining three groups were received different concentrations of RJ (50, 100 and 200 mg/kg/day) respectively. The orally dosages of RJ were determined according to Galaly *et al*. (2014) (12). 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 (13).

Sample collection

At the end of the experimental period, all rats were weighed. They were anesthetized, using a mixture of ketamine and xylazine i.m., and then they were sacrificed (14). The testes were removed, cleaned and fixed immediately in 10% formalin solution for later histological preparation

Histological technique

At the end of the experimental period (60 days), all rats anesthetized using a mixture of ketamine and xylazine i.m., and then they were sacrificed (14).Ordinary histological technique was followed to prepare slides from specimens of testes from all animal groups to study the alterations that may be found in treated animal groups when compared to control groups. The processing and staining techniques were performed according to Bancroft and Stevens (1982) (15).

Histopathological examination

The stained sections of testes from each animal were observed under the compound microscope to evaluate the histological features and structural changes. Photomicrographs were taken by camera mounted microscope at different magnifications.

Results and Discussion:

The microscopic examination of testicular sections of control rats showed normal histological structure. Photomicrographs of testes showed closely packed seminiferous tubules with little spaces between them in addition to obvious successive spermatogenic stages. Clumps of Leydig cells were obvious in the sections inside the interstitial spaces (Figures1&2).

In aluminum-treated group the histological appearance of testis after 60 days of exposure showed structural alterations as compared to the control group. The main histopathological changes were vacuolar degeneration of spermatogenic cells and Sertoli cells with clear necrotic debris in the seminiferous tubules which attained different shapes, appearance of multinucleated giant cells and/or pyknotic cells, irregularity of germ cell layers, abnormal distribution of spermatozoa in the lumens or no sperms in the lumen, thick and irregular basement membrane, interstitial edema, hyperplasia of Leydig cells, congestion of blood vessels, and intertubular space expansion (Figures 3&4).

On the contrary, the results of this study demonstrated that administration of RJ at graduated concentrations together with $AlCl_3$ result in progress enhancement in histologic testicular structure. Serious deleterious effects of $AlCl_3$ on testicular structure were reversed by RJ administration.

The treatment of male rats with RJ at the dose 50 mg/kg showed continuous effectual damage induced by $AlCl_3$ on the testis tissue (Figures 5&6), while the using of RJ at 100 mg/kg showed mild improvement in histologic feature with occurrence of marked edematous fluid and spermatogenic arrest at spermatids stage (Figures 7&8). The enhancement was evident at higher dose level of RJ (200 mg/kg) which include the restore arrangement of seminiferous tubules with apparently normal distribution of cellular elements. Also, sperm bundles retain to present in lumen of tubules (Figures 9 & 10).

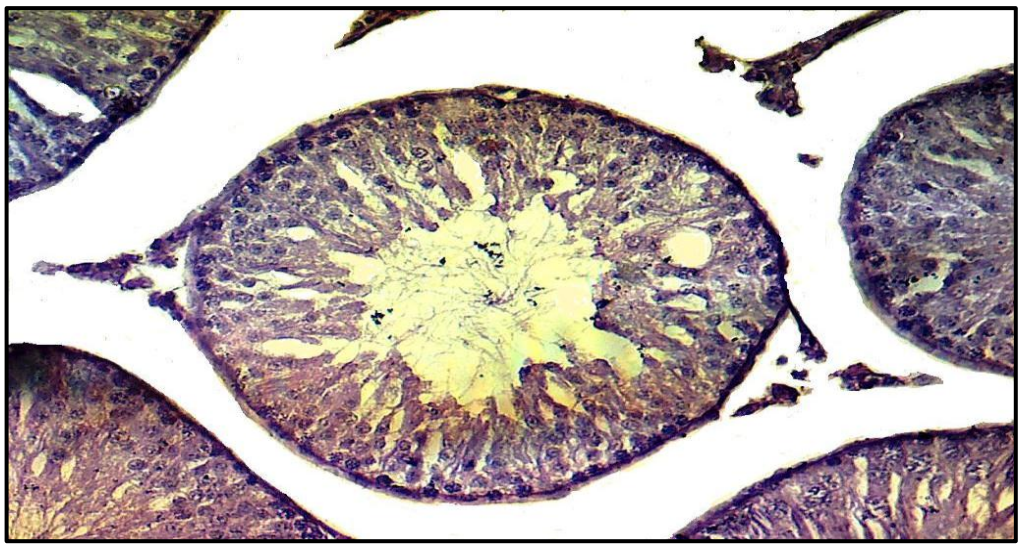

Figure (1): Photomicrograph of testis section from control group showing closely packed seminiferous tubules with little interstitial connective tissue and normal distribution of cellular element. (H & E, 100×).

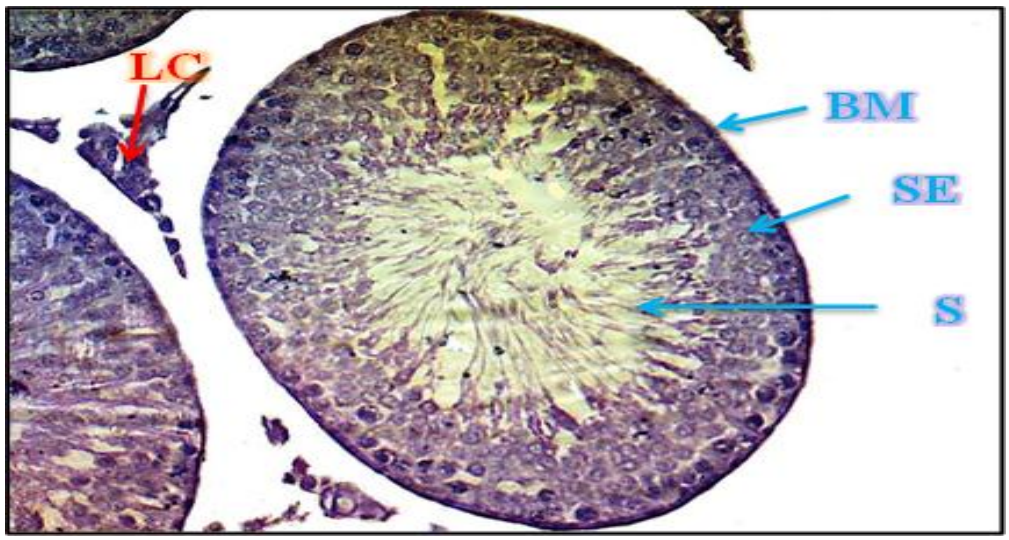

Figure (2): Photomicrograph of testis section from control group showing spermatogenic epithelium (SE), spermatozoa (S), Leydig cells (LC) and basement membrane (BM). (H & E, 200×).

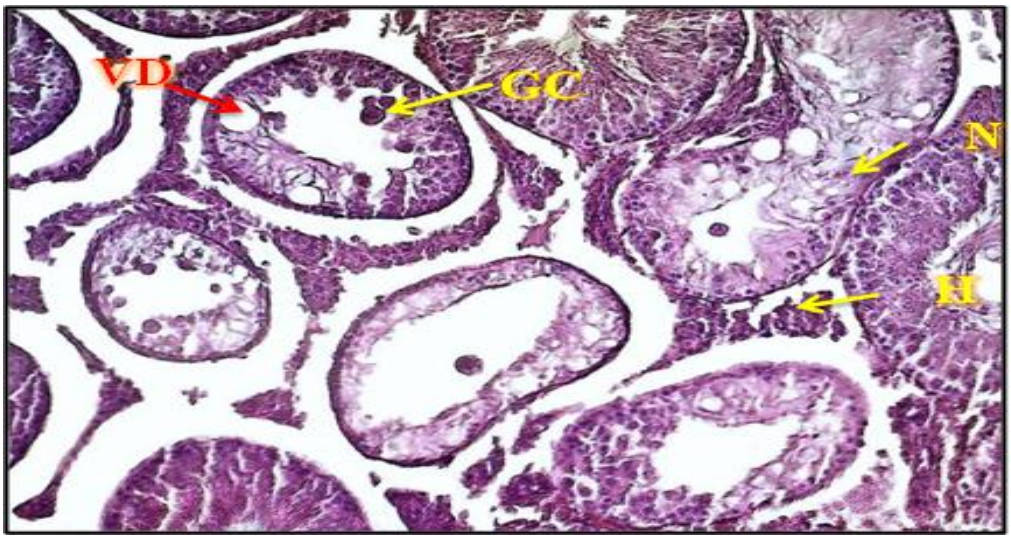

Figure (3): Photomicrograph of testis section from $AlCl_3$ -treated group (20 mg/kg) showing severe damage within the seminiferous tubules with inhibition of spermatogenesis. Note: vacuolar degeneration (VD) and necrosis (N) of germinal epithelium, formation of multinucleated giant cells (GC), and hyperplasia (H) of interstitial Leydig cells. (H & E, 200×).

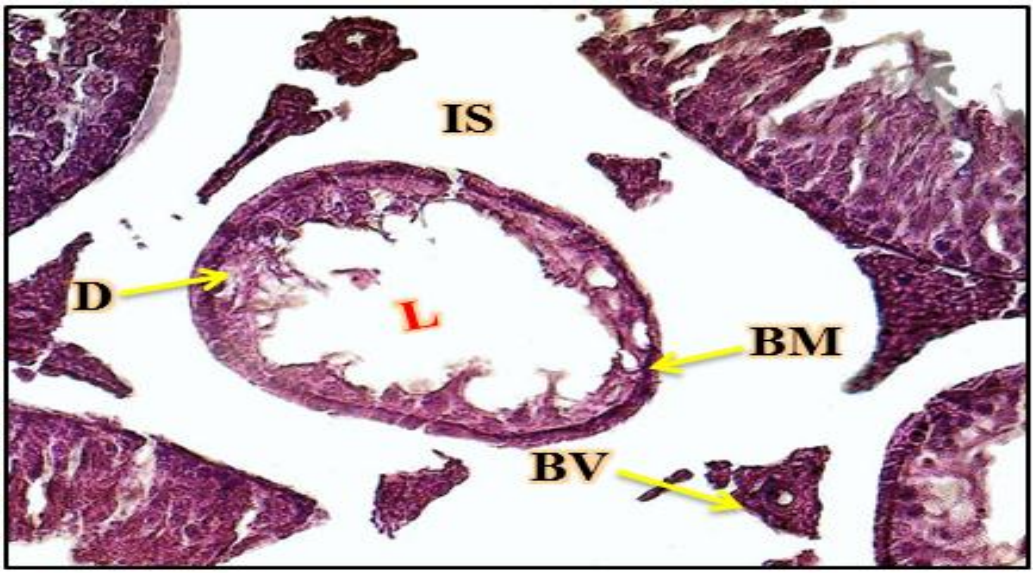

Figure (4): Photomicrograph of testis section from $AlCl_3$ -treated group (20 mg/kg) showing complete lesion in seminiferous tubule. Note: Degeneration (D) of spermatogenic cells and absence of spermatozoa, increase in the size of lumen (L), thick basement membrane (BM), increase in interstitial space (IS), and congested thick-walled blood vessels (BV). (H & E, 200×).

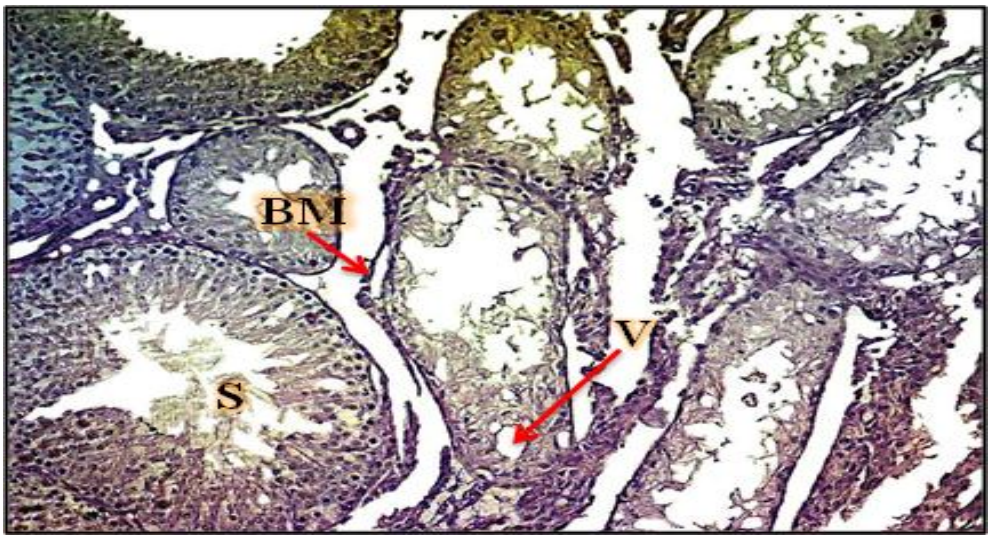

Figure (5): Photomicrograph of testis section from RJ-treated group (50 mg/kg) showing alterations of the general architecture in some seminiferous tubules. Note: disorganization of the germinal epithelium and occurrence of several large vacuoles (V), spermatogenesis arrest in some tubules and few fragmented spermatozoa (s) in the lumen, and irregular basement membrane (BM) . (H & E, 100×).

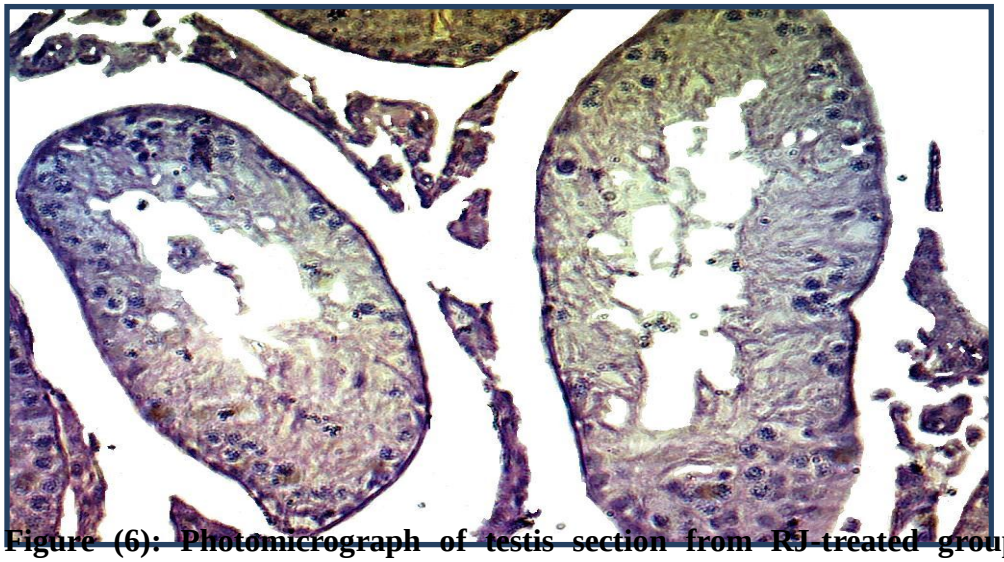

Figure (6): Photomicrograph of testis section from RJ-treated group (50 mg/kg) showing serious deleterious effects on testicular structure. Note: Hypoplasia (H) of the germinal epithelium with spermatogenic arrest at various stages of spermatogenesis, and marked increase in the intertubular spaces (IS). (H & E, 100×).

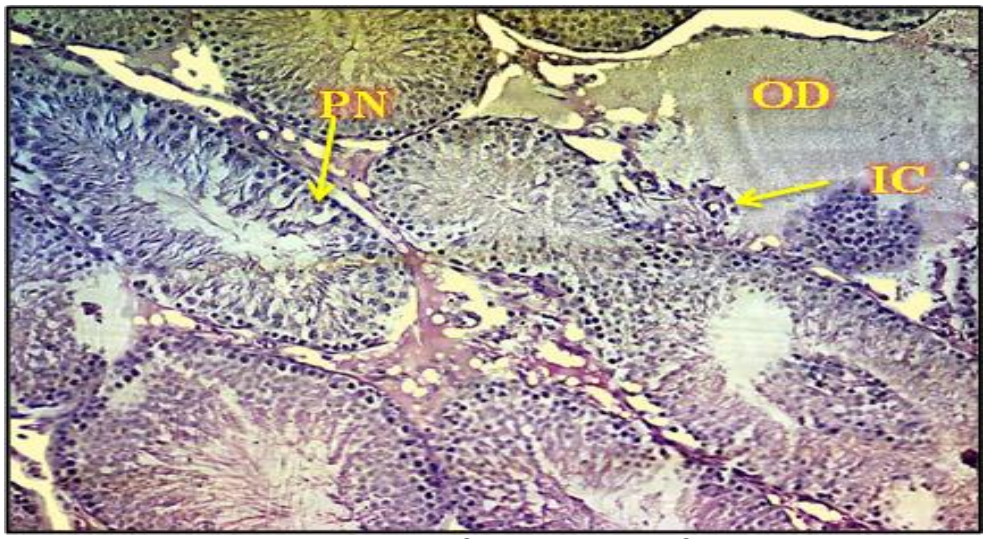

Figure (7): Photomicrograph of testis section from RJ-treated group (100 mg/kg) showing mild histological changes in the seminiferous tubules. Note: most of spermatogonia had pyknotic nuclei (PN), presence of homogenous acidophilic exudates or interstitial oedematous fluid (OD), and increase the inflammatory cells (IC) in the interstitium. (H & E, 100×).

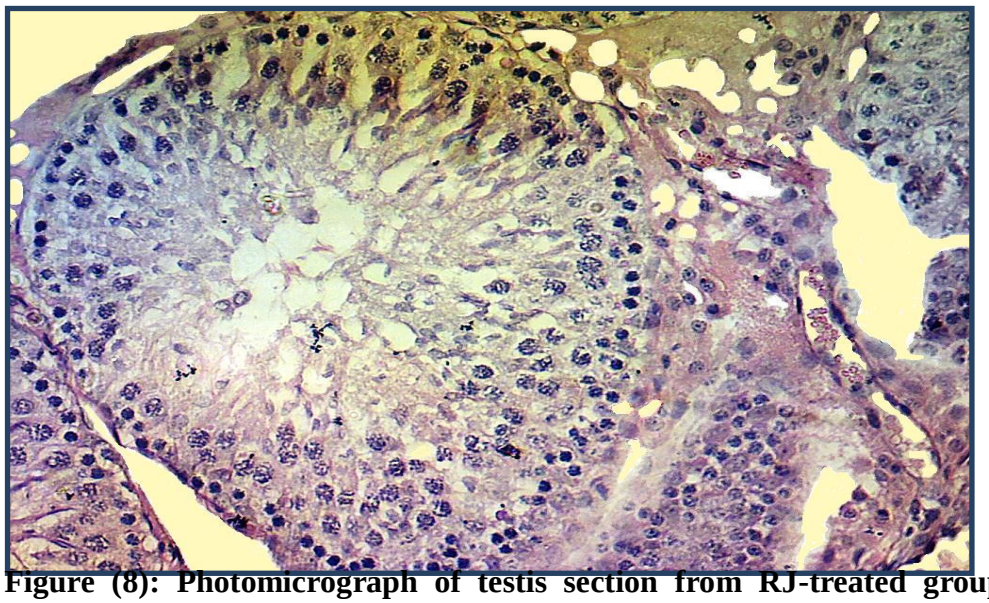

Figure (8): Photomicrograph of testis section from RJ-treated group (100 mg/kg) showing partial improvement in histologic feature. Note: arrest at the spermatid level in seminiferous tubules, arrest at the spermatid level in seminiferous tubule, interstitial edema and dilatation (D) of blood vessel. (H & E, 200×).

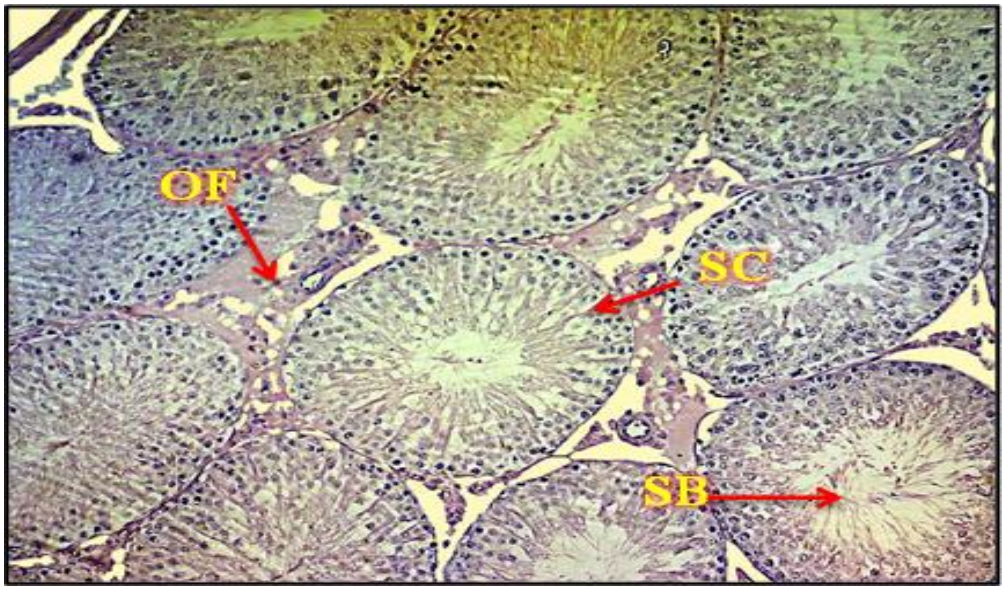

Figure (9): Photomicrograph of testis section from RJ-treated group (200 mg/kg) showing marked enhancement histological feature of seminiferous tubules. Note: different stages of normal spermatogenic cells (SC) with presence of sperm bundles (SB), and few areas of oedematous fluid (OF). (H & E, 200×).

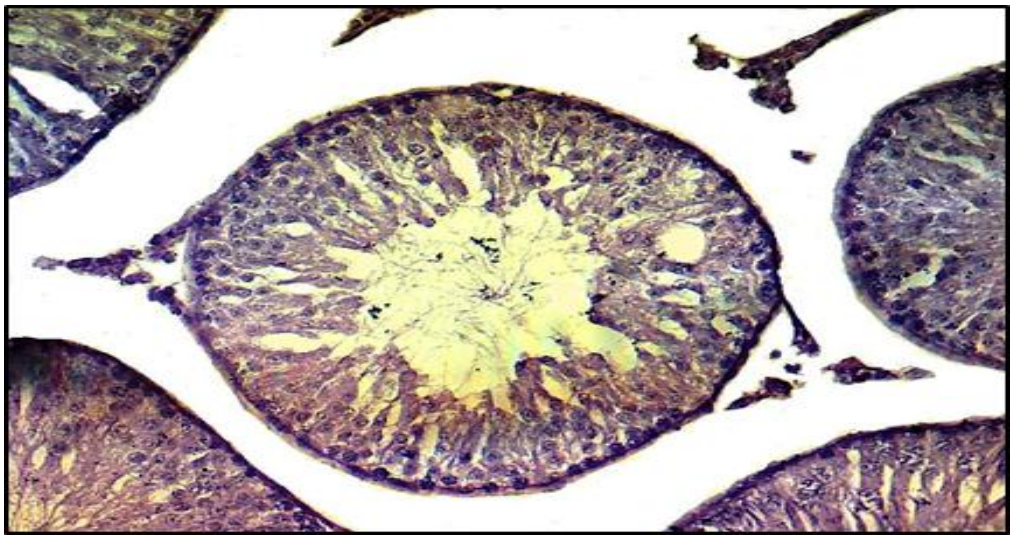

Figure (10): Photomicrograph of testis section from RJ-treated group (200 mg/kg) showing successive stages of normal spermatogenic cells of seminiferous tubule and normal distribution of epithelial lining. Damage reversed by RJ administration. (H & E, 200×).

Several studies performed on the human and experimental animals referred to the presence relationship between aluminum exposure and male reproductive toxicity. According to El-Demerdash *et al*. (2004) (16), the exposure to aluminum is responsible for increased oxidative stress and ROS which cause damage of cellular lipids, proteins and DNA. Consequently, these alterations might be representing the cause of degeneration and necrosis that revealed in the histology of testis.

Moreover, the cell membrane contains several highly unsaturated fatty acids which are easily attacked by free radicals leading to imitation lipid peroxidation which considered the rout for many pathological events (17). Furthermore, exposure to $AlCl_3$ may lead to a disorder in testosterone function resulting in deterioration in the spermatogenesis process in $AlCl_3$-treated group (18).

In the present work, the microscopic examination of aluminum-treated rats revealed the occurrence of multinucleated giant cells in the lumen of some tubules. Chinoy *et al*. (2005) (19) suggested that giant cells could be the result of faulty or failed chromosomal replication or cell division.

The hyperplasia of interstitial Leydig cells can be as a compensatory attempt in order to face the decline which occurred in testosterone level. This may result in alteration of GnRH levels which may affect LH secretion from the anterior pituitary lobe that stimulates Leydig cells to proliferate as a compensatory mechanism (20).

Presence of interstitial edema may result from the oxidative damage of capillaries endothelial cells through interference with their membranes, increasing their permeability (21). It was also reported that capillary filtration participates in interstitial fluid formation; thus, changes in capillary permeability and blood/lymph flow could modify the interstitial fluid (22). In addition, one study has reported that the congestion in blood vessel walls is attributed to hypertension in the surface blood vessels (23)

On the basis of our findings, histological observations in the present study proved the excellent recovery of testes after RJ treatment and this protection was dose dependent. RJ was used in this experiment in order to reverse or prevent toxic effect induced by aluminum based on some information which considered the RJ as a safe product when administrated to male rats and mice and did not lead to any adverse effect (24).

The present study has established the ameliorating effect of RJ in the testicular structure and spermatogenesis process. The therapeutic efficacy of RJ may be attributed to its antioxidant activity against lipid peroxidation and free radical generation.

The beneficial biological properties of RJ components exhibit an antioxidant capacity and improved endogenous antioxidant defense system, thereby, histopathologic picture of testes were reversed by treatment with RJ to normal architecture. These results are in agreement with many reports (25, 26, 27).

Conclusion:

This study showed that RJ has alleviating effect and protective role in testicular tissue, in the condition of aluminium-induced oxidative stress. Moreover, the RJ at concentration 200 mg/kg of body weight could be able to alleviate the disturbing effects of $AlCl_3$ and improved sexual efficiency of male rats.

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