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Research Article

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The attenuating effect of Royal Jelly on Hormonal Parameters in Aluminum Chloride (AlCl3) Intoxicated Rats

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ABSTRACT

The aim of this study is to investigate the protective effect of royal jelly (RJ) against Aluminum Chloride (AlCl3) toxicity on Pituitary, Thyroid and Sex Hormones in addition to histological sections of testis. The animals were given a dose of Aluminum Chloride (AlCl3) (30mg/kg) every other day intraperitoneally for eight weeks, and a dose of Royal jelly (RJ) (400mg/kg) daily in drinking water for eight weeks. Follicle stimulating hormone (FSH), luteinizing hormone (LH), thyroid stimulating hormone (TSH), thyroxin (T4), triiodothyronine (T3), percentage of triiodothyronine to thyroxin (T3/T4) and testosterone level were measured in blood serum. AlCl3 caused a significant decrease of FSH, LH, TSH, T4, T3, T3/T4 and testosterone, and caused the development of oligospermia, hypoplasia, congested blood vessel and exfoliated tubules in the testis, but royal jelly attenuates these effects.
Key words: *Aluminum chloride (AlCl3), royal jelly, pituitary, thyroid, sex Hormones, follicle stimulating hormone (FSH), luteinizing hormone (LH), thyroid stimulating hormone (TSH), thyroxin (T4), triiodothyronine (T3), percentage of triiodothyronine to thyroxin (T3/T4), testosterone, testes, histology.*

INTRODUCTION

Aluminum metal is abundantly present in the earth's crust. From the environment it gets access to the human body via the gastrointestinal and the respiratory tracts. Aluminum is a constituent of cooking utensils and medicines such as antacids, deodorants and food additives and this has allowed its easy access into the body [1]. The sources of aluminum are especially corn, yellow cheese, salt, herbs, spices, tea, cosmetics, ware and containers. Also, it is present in medicines and is also added to drinking water for purification purposes [2]. Aluminum absorption/accumulation in humans can occur via the diet and drinking water, ingestion of fruit juices with citric acid causes a marked increase in both gastrointestinal absorption and urinary excretion of aluminum in healthy subjects [3]. It has been proposed as an environmental factor that may contribute to some neurodegenerative diseases, and affects several enzymes and other biomolecules relevant to Alzheimer's disease [4]. Aluminum chloride (AlCl3) has been reported to induce oxidative damage and to inhibit the activities of antioxidant enzymes [5].

Royal jelly (RJ), a honey bee secretion used in the nutrition of larvae, as well as adult queens is secreted from the glands in the hypopharynx of worker bees, and fed to all larvae in the colony [6]. It is collected and sold as a dietary supplement, claiming various health benefits because of components like B-complex vitamins such as pantothenic acid (vitamin B5) and vitamin B6 (pyridoxine). The overall composition of royal jelly is 67% water, 12.5% crude

protein (including small amounts of many different amino acids), and 11% monosaccharides, and a relatively high amount (5%) of fatty acids. It also contains many trace minerals, some enzymes and trace amounts of vitamin C [7]. Royal jelly is a time-honored Chinese remedy known to have numerous functions, such as maintenance of health. Furthermore, RJ functions as an antimicrobial [8] and antitumor [9]. Royal jelly is capable of exhibiting potential immunomodulation in mice by stimulating antibody production and immunocompetent cell proliferation [10]. Proteins and peptides of RJ have many effects such as antioxidative [11], monocyte-proliferation stimulating [12], anti-inflammatory [13].

MATERIALS AND METHODS

1) Aluminum chloride (AlCl3):
The animals will be given a dose of 30mg/kg every other day intraperitoneally for eight weeks [14].

2) Royal jelly (RJ):

The animals were given a dose of 400mg/kg daily in drinking water for eight weeks [15].

The animals to be sampled were weighed and they are 36 animals as of 6 animals from each group. Blood sampling was immediately after slaughter the testes was preserved in neutral formalin solution until histological section were made.

The serum was collected in small glass bottles for each animal and were kept in the freezer at -18°C to be used for biochemical measurements.

- The pituitary gland hormones (follicle stimulating hormone (FSH), luteinizing hormone (LH), thyroid stimulating hormone (TSH)), measuring thyroid gland hormones level (thyroxin (T4), triiodothyronine (T3), percentage of triiodothyronine to thyroxin T4/T3), and testosterone level were measured.

Statistical study:

Mean will be calculated for control group and treated groups, also the standard deviation S.E. for means and T-Test at a 5% significant level [16].

RESULTS

1) Follicle stimulating hormone (FSH):

Aluminum chloride group showed a significant decrease in FSH from week 4 to 8 compared to control, but did not show any significant difference in FSH in the first 3 weeks compared to control. A significant increase in FSH was noted in the royal jelly group from week 4 to 8 compared to AlCl3 group except of week 4 which had no significant difference, and no significant difference was noted in the first 3 weeks compared to control and AlCl3 group. Aluminum chloride+royal jelly group showed a significant increase in week 2 and 5 to 8 compared to AlCl3 group, and significant increase in week 7 compared to control, but no significant difference in FSH in week 1, 3 and 4 compared to control and AlCl3 group (Table 1).

Table (1): Effect of daily administration of Royal jelly (400 mg/kg), Aluminum chloride (30 mg/kg) and combination of Royal jelly and Aluminum chloride on follicle stimulating hormone (FSH) (mIu/ml) of adult albino rats.

	Control	AlCl3	Royal Jelly	AlCl3+ Royal Jelly
	Mean ± S.E	Mean ± S.E	Mean ± S.E	Mean ± S.E
Week1	3.53 ± 0.33	3.54 ± 0.29 0.47 %	3.22 ± 0.21 -8.75 %	3.68 ± 0.17 4.49 %

Week2	3.77 ± 0.31	3.63 ± 0.34 -3.54 %	4.17 ± 0.35 10.62 %	4.63 ± 0.30 b 23.01 %
Week3	3.67 ± 0.28	3.32 ± 0.21 -9.55 %	3.40 ± 0.19 -7.27 %	3.43 ± 0.22 -6.36 %
Week4	4.09 ± 0.30	3.22 ± 0.20 a -21.29 %	3.95 ± 0.27 -3.34 %	3.93 ± 0.31 -3.75 %
Week5	3.97 ± 0.23	2.80 ± 0.17 a -29.41 %	3.77 ± 0.29 b -5.04 %	4.52 ± 0.42 b 13.87 %
Week6	4.08 ± 0.34	2.92 ± 0.14 a -28.57 %	4.95 ± 0.37 b 21.22 %	4.60 ± 0.32 b 12.65 %
Week7	4.17 ± 0.11	3.15 ± 0.22 a -24.40 %	4.18 ± 0.25 b 0.40 %	5.38 ± 0.34 a,b 29.20 %
Week8	4.12 ± 0.36	2.68 ± 0.07 a -34.82 %	4.58 ± 0.39 b 11.34 %	4.57 ± 0.25 b 10.93 %

a Significant difference from the control group at $p < 0.05$.b Significant difference from the AlCl3-intoxicated group at $p < 0.05$.
statistical analysis was performed between control (C=6) and treated (T=6) using T-Test

2) Luteinizing hormone (LH):

Aluminum chloride group showed a significant decrease in LH from week 3 to 8 compared to control, but did not show any significant difference in LH in the first 2 weeks. There was a significant increase in the LH of royal jelly group and AlCl3 +royal jelly group from week 3 to 8 compared to AlCl3 group, but no significant difference was noted in the LH in the first 2 weeks compared to control and AlCl3 group (Table 2).

3) Testosterone:

Aluminum chloride group showed a significant decrease in testosterone from week 2 to 8 compared to control, but did not show any significant difference in testosterone in the first week. A significant increase was noted in the testosterone of royal jelly group in all 8 weeks compared to AlCl3 group. A significant increase was seen in all 8 weeks except the first week in the AlCl3+royal jelly group compared to AlCl3 group, and from week 5 to 8 compared to control, and no significant change in testosterone was seen in the first week compared to control and AlCl3 group (Table 3).

4) Thyroid stimulating hormone (TSH):

Aluminum chloride group showed a significant decrease in TSH from week 3 to 8 compared to control, but did not show any significant difference in TSH in the first 2 weeks. A significant increase in TSH was noted in royal jelly group and AlCl3+royal jelly group from week 3 to 8 compared to AlCl3 group, but no significant difference was noted in the TSH in the first 2 weeks compared to control and AlCl3 group (Table 4).

Table (2): Effect of daily administration ofRoyal jelly (400 mg/kg), Aluminum chloride (30 mg/kg) and combination of Royal jelly and Aluminum chloride on luteinizing hormone (LH) (mIu/ml) of adult albino rats.

	Control Mean ± S.E	AlCl3 Mean ± S.E	Royal Jelly Mean ± S.E	AlCl3+ Royal Jelly Mean ± S.E
Week1	5.18 ± 0.39	4.93 ± 0.14 -4.82 %	5.37 ± 0.37 3.54 %	4.33 ± 0.36 -16.53 %
Week2	5.08 ± 0.36	5.30 ± 0.27 4.26 %	5.32 ± 0.34 4.59 %	4.63 ± 0.35 -8.85 %
Week3	5.82 ± 0.40	4.20 ± 0.24 a -27.79 %	5.73 ± 0.37 b -1.43 %	5.05 ± 0.27 b -13.18 %

Week4	5.63 ± 0.36	3.73 ± 0.27 a -33.73 %	5.35 ± 0.35 b -5.03 %	4.85 ± 0.16 b -13.91 %
Week5	5.53 ± 0.20	3.73 ± 0.29 a -32.68 %	5.38 ± 0.29 b -2.71 %	4.98 ± 0.30 b -9.94 %
Week6	6.28 ± 0.17	3.80 ± 0.28 a -39.52 %	6.27 ± 0.47 b -0.27 %	6.38 ± 0.36 b 1.59 %
Week7	5.15 ± 0.48	3.38 ± 0.22 a -34.30 %	5.27 ± 0.36 b 2.27 %	5.37 ± 0.30 b 4.21 %
Week8	5.45 ± 0.46	3.93 ± 0.03 a -27.83 %	5.18 ± 0.39 b -4.89 %	5.22 ± 0.49 b -4.28 %

a Significant difference from the control group at $p < 0.05$.b Significant difference from the AlCl3-intoxicated group at $p < 0.05$.
statistical analysis was performed between control (C=6) and treated (T=6) using T-Test

Table (3): Effect of daily administration of Royal jelly (400 mg/kg), Aluminum chloride (30 mg/kg) and combination of Royal jelly and Aluminum chloride on testosterone (ng/ml) of adult albino rats.

	Control	AlCl3	Royal Jelly	AlCl3+ Royal Jelly
	Mean ± S.E	Mean ± S.E	Mean ± S.E	Mean ± S.E
Week1	4.00 ± 0.33	3.71 ± 0.20 -7.23 %	4.31 ± 0.12 b 7.90 %	4.38 ± 0.39 9.63 %
Week2	4.97 ± 0.44	2.80 ± 0.16 a -43.66 %	4.92 ± 0.27 b -0.95 %	4.05 ± 0.27 b -18.55 %
Week3	4.26 ± 0.39	2.63 ± 0.12 a -38.15 %	4.10 ± 0.18 b -3.71 %	3.84 ± 0.24 b -9.84 %
Week4	4.86 ± 0.17	2.78 ± 0.05 a -42.77 %	4.43 ± 0.12 b -8.72 %	4.60 ± 0.30 b -5.41 %
Week5	4.56 ± 0.38	3.37 ± 0.26 a -26.18 %	4.77 ± 0.20 b 4.52 %	6.85 ± 0.07 a,b 50.23 %
Week6	4.94 ± 0.19	2.95 ± 0.17 a -40.29 %	4.48 ± 0.14 b -9.42 %	6.82 ± 0.11 a,b 37.95 %
Week7	4.86 ± 0.32	2.09 ± 0.02 a -57.10 %	4.17 ± 0.34 b -14.21 %	6.72 ± 0.11 a,b 38.37 %
Week8	4.69 ± 0.26	2.25 ± 0.16 a -52.02 %	4.34 ± 0.29 b -7.61 %	6.65 ± 0.17 a,b 41.64 %

a Significant difference from the control group at $p < 0.05$.b Significant difference from the AlCl3-intoxicated group at $p < 0.05$.
statistical analysis was performed between control (C=6) and treated (T=6) using T-Test

Table (4): Effect of daily administration ofRoyal jelly (400 mg/kg), Aluminum chloride (30 mg/kg) and combination of Royal jelly and Aluminum chloride on thyroid stimulating hormone (TSH) (mIu/l) of adult albino rats.

	Control	AlCl3	Royal Jelly	AlCl3+ Royal Jelly
	Mean ± S.E	Mean ± S.E	Mean ± S.E	Mean ± S.E
Week1	1.19 ± 0.05	1.20 ± 0.10 0.84 %	1.11 ± 0.06 -6.86 %	1.05 ± 0.07 -12.04 %
Week2	1.04 ± 0.04	1.05 ± 0.09 0.80 %	1.13 ± 0.01 8.33 %	1.02 ± 0.07 -2.02 %

Week3	1.58 ± 0.07	1.06 ± 0.02 a -33.26 %	1.35 ± 0.11 b -15.05 %	1.40 ± 0.12 b -11.89 %
Week4	1.10 ± 0.04	0.71 ± 0.06 a -35.80 %	1.11 ± 0.04 b 0.76 %	1.20 ± 0.07 b 8.46 %
Week5	1.25 ± 0.03	0.75 ± 0.03 a -39.89 %	1.20 ± 0.05 b -3.61 %	1.14 ± 0.06 b -8.17 %
Week6	1.07 ± 0.09	0.50 ± 0.02 a -52.89 %	1.27 ± 0.11 b 18.72 %	1.12 ± 0.09 b 5.15 %
Week7	1.10 ± 0.08	0.77 ± 0.01 a -29.38 %	1.07 ± 0.02 b -1.98 %	1.07 ± 0.05 b -2.59 %
Week8	1.32 ± 0.10	0.86 ± 0.03 a -34.98 %	1.25 ± 0.10 b -4.82 %	1.28 ± 0.10 b -2.53 %

a Significant difference from the control group at $p < 0.05$.b Significant difference from the AlCl3-intoxicated group at $p < 0.05$.
statistical analysis was performed between control (C=6) and treated (T=6) using T-Test

5) Thyroxine (T4):

Aluminum chloride group showed a significant decrease in T4 from week 4 to 8 compared to control, but did not show any significant difference in T4 in the first 3 weeks. A significant increase in T4 of royal jelly group was noted from week 1 to 8 compared to AlCl3 group, and a significant increase in week 8 in compared to control. AlCl3+royal jelly group showed a significant increase in week 1, 2, 3, 4 and 8 compared to control, and a significant increase in all 8 weeks except for the sixth week compared to AlCl3 group, and showed no significant difference in T4 in the sixth week compared to control and AlCl3 group (Table 5).

6) Triiodothyronine (T3):

Aluminum chloride group showed a significant decrease in T3 in all 8 weeks of experiment except for the first week compared to control, and did not show any significant difference in T3 in the first week. A significant increase in T3 was noted in royal jelly group and AlCl3+royal jelly group in week 2 to 8 compared to AlCl3 group. A significant decrease was noted in AlCl3+royal jelly group in the second week compared to control (Table 6).

7) Triiodothyronine to thyroxine ratio (T3/T4):

Aluminum chloride group showed a significant decrease in T3/T4 in week all 8 weeks except for the first week compared to control, but did not show any significant difference in T3/T4 in the first week. There was a significant decrease in T3/T4 of the royal jelly group in the first three weeks compared to control and AlCl3 group, and there was a significant decrease in the fourth week compared to control group, and there was a significant increase from week 5 to 8 compared to AlCl3 group. Aluminum chloride+royal jelly group showed a significant decrease in T3/T4 in the first four weeks compared to control and AlCl3 group, and a significant increase in the last four weeks compared to AlCl3 group (Table 7).

Table (5): Effect of daily administration ofRoyal jelly (400 mg/kg), Aluminumchloride (30 mg/kg) and combination of Royal jelly and Aluminumchloride on thyroxine hormone (T4) (ng/ml)of adultalbino rats.

	Control Mean± S.E	$AlCl_3$ Mean± S.E	Royal Jelly Mean± S.E	$AlCl_3$+ Royal Jelly Mean± S.E
Week1	2.70 ± 0.19	2.32 ± 0.11 -13.94 %	7.22 ± 0.46[a,b] 167.69 %	6.92 ± 0.34[a,b] 156.85 %
Week2	3.50 ± 0.33	3.58 ± 0.30 2.22 %	6.83 ± 0.32[a,b] 95.11 %	7.85 ± 0.66[a,b] 124.12 %
Week3	6.57 ± 0.57	5.22 ± 0.37 -20.64 %	9.49 ± 0.73[a,b] 44.35 %	10.24 ± 0.49[a,b] 55.76 %
Week4	6.98 ± 0.20	4.55 ± 0.21 [a] -34.79 %	8.46 ± 0.70 [b] 21.25 %	14.98 ± 0.42[a,b] 114.67 %
Week5	8.83 ± 0.56	6.22 ± 0.54 [a] -29.57 %	8.41 ± 0.78 [b] -4.85 %	9.60 ± 0.84 [b] 8.62 %

Week6	10.02 ± 0.59	7.69 ± 0.52 [a] -23.32 %	9.71 ± 0.28 [b] -3.09 %	8.45 ± 0.59 -15.69 %
Week7	9.84 ± 0.29	7.91 ± 0.12 [a] -19.70 %	10.78 ± 0.80 [b] 9.52 %	9.45 ± 0.23 [b] -4.02 %
Week8	7.17 ± 0.18	5.53 ± 0.47 [a] -22.92 %	8.55 ± 0.50[a,b] 19.26 %	11.18 ± 0.88[a,b] 55.95 %

[a]Significantdifferencefrom the control group at $p < 0.05$.[b]Significantdifferencefrom the $AlCl_3$-intoxicated group at $p < 0.05$.
statisticalanalysiswasperformedbetween control (C=6) and treated (T=6) using T-Test

Table (6): Effect of daily administration of Royal jelly (400 mg/kg), Aluminumchloride (30 mg/kg) and combination of Royal jelly and Aluminumchloride on triiodothyronine (T3) (ng/ml)of adultalbino rats.

	Control Mean± S.E	$AlCl_3$ Mean± S.E	Royal Jelly Mean± S.E	$AlCl_3$+ Royal Jelly Mean± S.E
Week1	1.58 ± 0.14	1.38 ± 0.13 -12.79 %	1.34 ± 0.10 -15.27 %	1.63 ± 0.13 3.11 %
Week2	1.90 ± 0.11	1.24 ± 0.09 [a] -34.96 %	1.72 ± 0.13 [b] -9.43 %	1.47 ± 0.03[a,b] -22.88 %
Week3	2.68 ± 0.15	1.72 ± 0.10 [a] -35.86 %	2.33 ± 0.16 [b] -12.99 %	2.25 ± 0.12 [b] -16.02 %
Week4	2.61 ± 0.16	1.33 ± 0.13 [a] -49.18 %	2.36 ± 0.10 [b] -9.46 %	2.46 ± 0.10 [b] -5.94 %
Week5	2.34 ± 0.13	0.68 ± 0.07 [a] -70.88 %	2.39 ± 0.11 [b] 2.25 %	2.35 ± 0.11 [b] 0.28 %
Week6	2.35 ± 0.12	0.94 ± 0.06 [a] -60.29 %	2.42 ± 0.09 [b] 2.67 %	2.15 ± 0.06 [b] -8.70 %
Week7	3.45 ± 0.15	1.80 ± 0.04 [a] -47.68 %	3.48 ± 0.13 [b] 1.04 %	3.41 ± 0.29 [b] -1.05 %
Week8	3.20 ± 0.21	1.43 ± 0.11 [a] -55.41 %	3.31 ± 0.09 [b] 3.47 %	3.44 ± 0.15 [b] 7.31 %

[a]Significantdifferencefrom the control group at $p < 0.05$.[b]Significantdifferencefrom the $AlCl_3$-intoxicated group at $p < 0.05$.
statisticalanalysiswasperformedbetween control (C=6) and treated (T=6) using T-Test

Table (7): Effect of daily administration ofRoyal jelly (400 mg/kg), Aluminumchloride (30 mg/kg) and combination of Royal jelly and Aluminumchloride on triiodothyronine to thyroxine ratio (T3/T4) (ng/ml) of adultalbino rats.

	Control Mean± S.E	$AlCl_3$ Mean± S.E	Royal Jelly Mean± S.E	$AlCl_3$+ Royal Jelly Mean± S.E
Week1	0.59 ± 0.019	0.59 ± 0.043 1.59 %	0.19 ± 0.004[a,b] -68.00 %	0.24 ± 0.013[a,b] -58.67 %
Week2	0.58 ± 0.022	0.36 ± 0.023 [a] -38.78 %	0.25 ± 0.012[a,b] -56.75 %	0.20 ± 0.011[a,b] -66.43 %
Week3	0.44 ± 0.028	0.33 ± 0.014 [a] -24.04 %	0.26 ± 0.010[a,b] -41.07 %	0.22 ± 0.005[a,b] -49.59 %
Week4	0.38 ± 0.022	0.30 ± 0.017 [a] -21.62 %	0.29 ± 0.011 [a] -23.47 %	0.17 ± 0.011[a,b] -56.23 %
Week5	0.27 ± 0.009	0.12 ± 0.002 [a] -57.44 %	0.30 ± 0.019 [b] 10.56 %	0.26 ± 0.012 [b] -5.24 %

Week6	0.24 ± 0.023	0.13 ± 0.008 [a] -47.82 %	0.25 ± 0.017 [b] 4.43 %	0.26 ± 0.015 [b] 9.60 %
Week7	0.35 ± 0.020	0.23 ± 0.007 [a] -35.10 %	0.33 ± 0.024 [b] -6.07 %	0.36 ± 0.017 [b] 3.31 %
Week8	0.45 ± 0.036	0.28 ± 0.017 [a] -38.18 %	0.40 ± 0.034 [b] -11.61 %	0.36 ± 0.018 [b] -19.94 %

[a]Significantdifferencefrom the control group at $p < 0.05$.[b]Significantdifferencefrom the $AlCl_3$-intoxicated group at $p < 0.05$.
statisticalanalysiswasperformedbetween control (C=6) and treated (T=6) using T-Test

Effect of Aluminum chloride (AlCl3) on histological structures of testis:

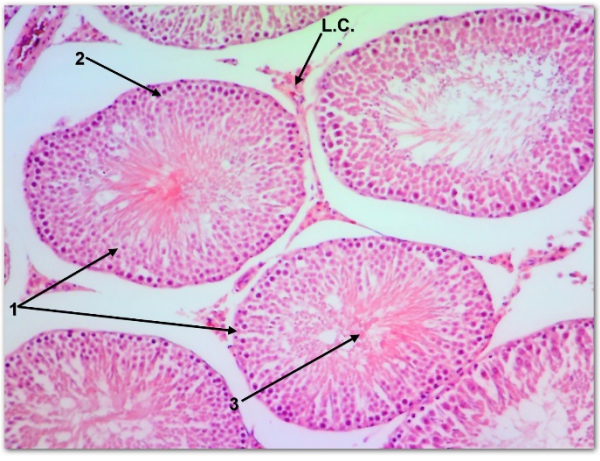
Fig. (1, 2) shows normal testis structure of control group. The study of histological structures of testis in the Aluminum chloride intoxicated rats showed that after two weeks of administration (Fig. 3, 4) the development of oligospermia, hypoplasia, congested blood vessel and exfoliated tubules but normal Leydig cells. After four weeks (Fig. 5, 6) the previous effects were increased and vascular degeneration was developed and Leydig cells were still normal. After six weeks (Fig. 7, 8) tubules showed increased oligospermia, exfoliated tubule with hypoplasia, increased spaces between tubules and degeneration of interstitial tissue. Eight weeks of administration (Fig. 9, 10) showed distorted tubule shape, lack of normal distribution of epithelial lining, increased space between tubules with degeneration of interstitial tissue, congested blood vessel and abnormal Leydig cells.

Effect of Royal jelly on histological structures of testis:

The testis tissue structure of the Royal jelly treated group (Fig. 11, 12) was studied and showed no change from the control group.

Effect of combination of Royal jelly and Aluminum chloride on histological structures of testis:

Studying the histological structures of testis of the AlCl3+Royal jelly group after two weeks (Fig. 13, 14) showed some tubules with hypoplasia and oligospermia, tubules with slight exfoliation and other tubules regaining their normal cell distribution with increase of sperm and normal leydig cells, there was also degeneration of interstitial tissue. After four weeks (Fig. 15, 16) there was tubules with less hypoplasia and oligospermia while some tubules had normal epithelial cell lining, normal sperms and normal leydig cells. After six weeks (Fig. 17, 18) there was some tubules with slight hypoplasia and oligospermia and more tubules with normal epithelial cell lining, increase of sperm and normal leydig cells. After eight weeks (Fig. 19, 20) there was less tubules with hypoplasia and oligospermia and tubules with normal epithelial cell lining, increase of sperm and normal leydig cells.



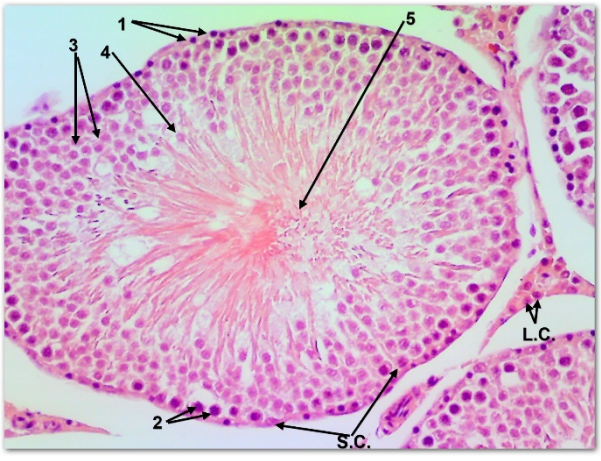


Fig. 1: A transverse section in rat testis from the control group shows normal seminiferous tubules (1), germinal epithelium (2) sperms (3) and Leydig cells (L.C.). X100, H&E.

Fig. 2: A transverse section in rat testis from the control group shows layers distribution of seminiferous epithelium cells in their different stages inside the seminiferous tubule: 1) Spermatogonia. 2) Primary spermatocytes. 3) Secondary spermatocytes. 4) Spermatids. 5) Mature Spermatozoa, Sertoli cells (S.C.) and Leydig cells (L.C.). X400, H&E.

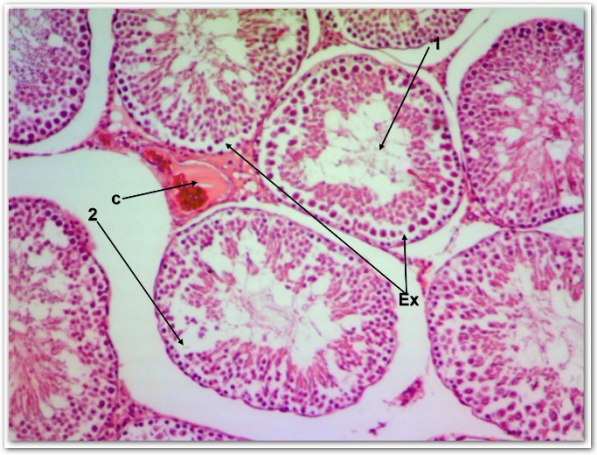


Fig. 3: A transverse section in rat testis from the two weeks $AlCl_3$ intoxicated group shows oligospermia (1), hypoplasia (2), congested blood vessel (C), exfoliated tubule (Ex) and decrease of Leydig cells (L.C.). X100, H&E.

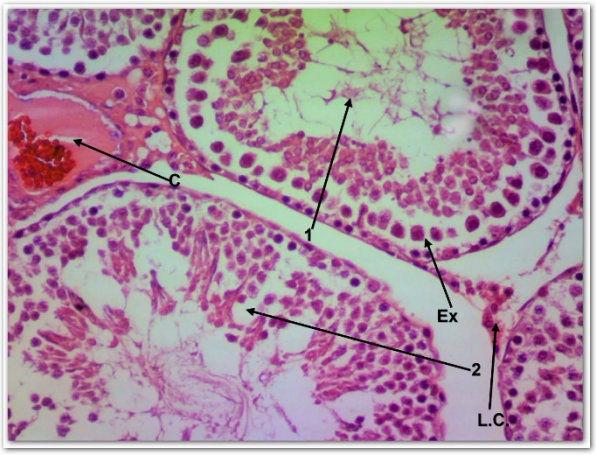


Fig. 4: A transverse section in rat testis from the two weeks $AlCl_3$ intoxicated group shows oligospermia (1), hypoplasia (2), congested blood vessel (C), exfoliated tubule (Ex) and decrease of Leydig cells (L.C.). X400, H&E.

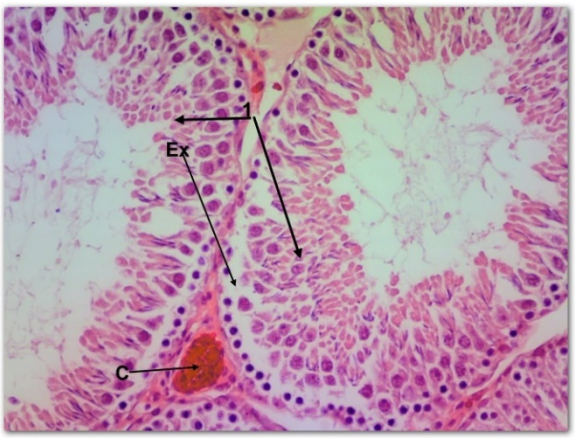


Fig. 5: A transverse section in rat testis from the four weeks $AlCl_3$ intoxicated group shows hypoplasia (1), congested blood vessel (C), exfoliated tubule (Ex), vascular degeneration (V.D.) and decrease of Leydig cells (L.C.). X400, H&E.

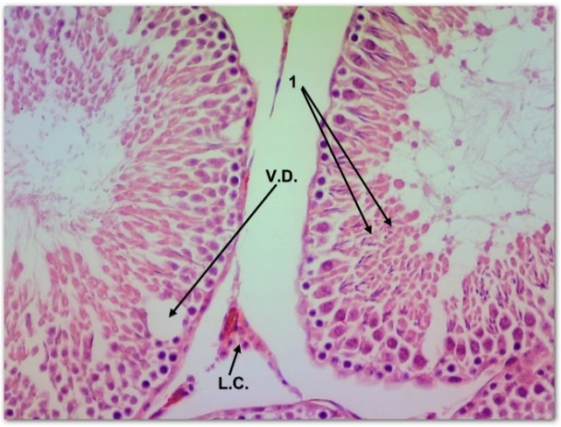


Fig. 6: A transverse section in rat testis from the four weeks $AlCl_3$ intoxicated group shows hypoplasia (1), congested blood vessel (C), exfoliated tubule (Ex), vascular degeneration (V.D.) and decrease of Leydig cells (L.C.). X400, H&E.

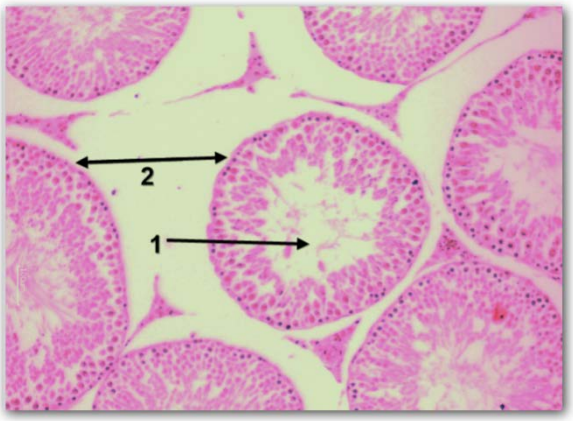


Fig. 7: A transverse section in rat testis from the six weeks $AlCl_3$ intoxicated group shows oligospermia (1) and increased spaces between tubules. X100, H&E.

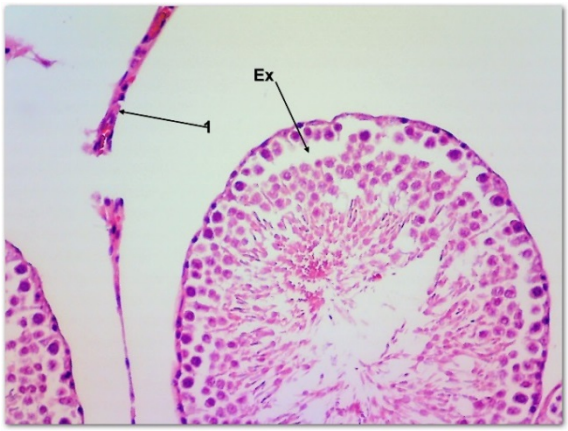


Fig. 8: A transverse section in rat testis from the six weeks $AlCl_3$ intoxicated group shows degeneration of interstitial tissue and decrease of Leydig cells (1) and exfoliated tubule (Ex) with hypoplasia. X400, H&E.

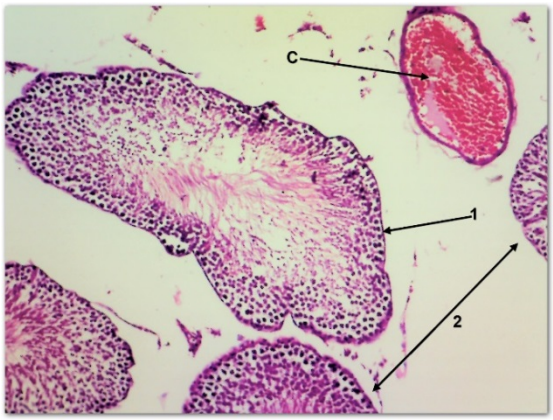


Fig. 9 : A transverse section in rat testis from the eightweeks $AlCl_3$intoxicated group shows distorted tubule shape (1), increasedspacebetween tubules withdegeneration of interstitial tissue (2) and congestedbloodvessel (C). X100, H&E.

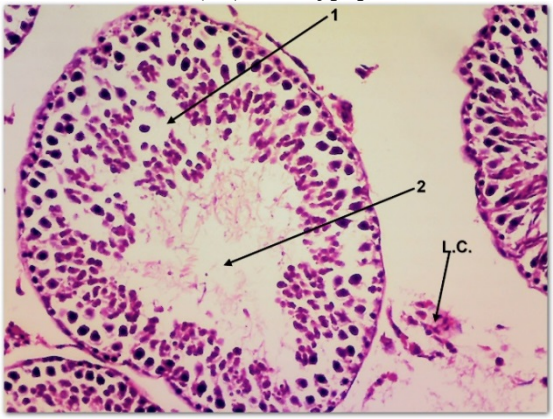


Fig. 10 : A transverse section in rat testis from the eightweeks $AlCl_3$intoxicated group shows lack of normal distribution of epitheliallining (1), oligospermia (2) and decrease of Leydigcells (L.C.). X400, H&E.

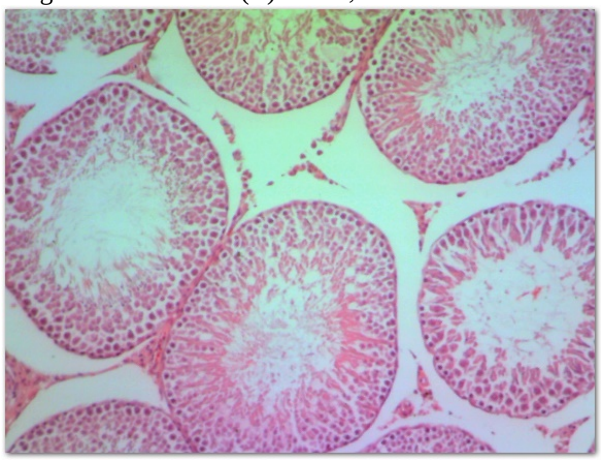


Fig. 11 : A transverse section in rat testis of the royal jellytreated group that shows normal tissue structure of testis. X100, H&E.

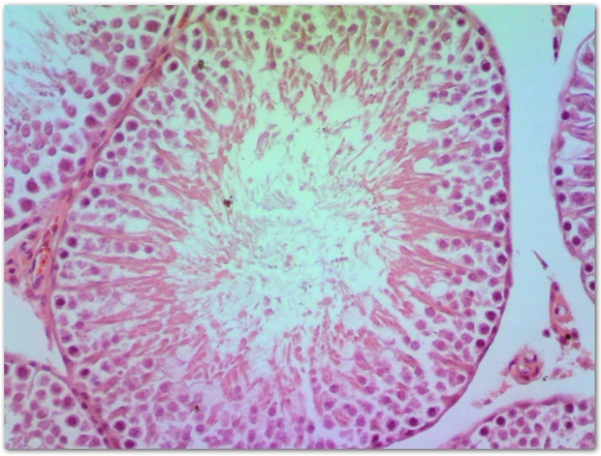


Fig. 12 : A transverse section in rat testis of the royal jellytreated group that shows normal tissue structure of testis. X400, H&E.

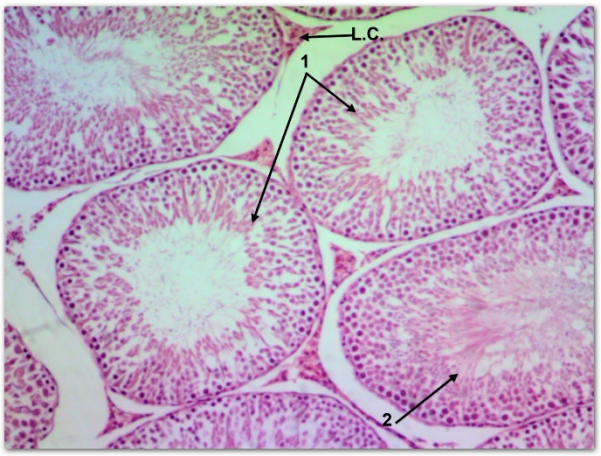


Fig. 13: A transverse section in rat testis from the two weeks $AlCl_3$+Royal jelly group shows tubules with hypoplasia and oligospermia (1), tubules regaining normal cell distribution and increase of sperm (2) and normal leydig cells (L.C.). X100, H&E.

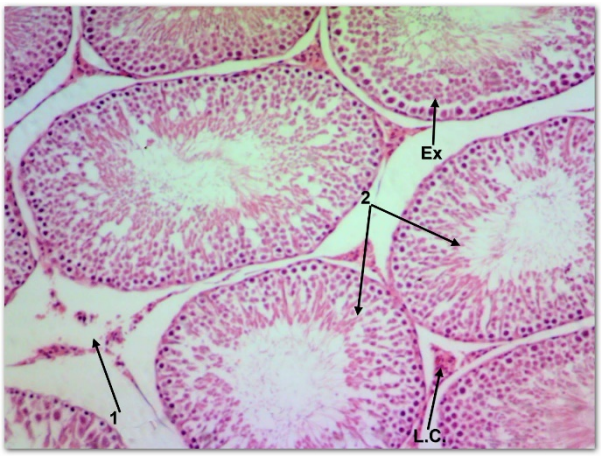


Fig. 14: A transverse section in rat testis from the two weeks $AlCl_3$+Royal jelly group shows degeneration of interstitial tissue (1), tubules with hypoplasia and oligospermia (2), slight exfoliated tubule (Ex) and normal leydig cells (L.C.). X100, H&E.

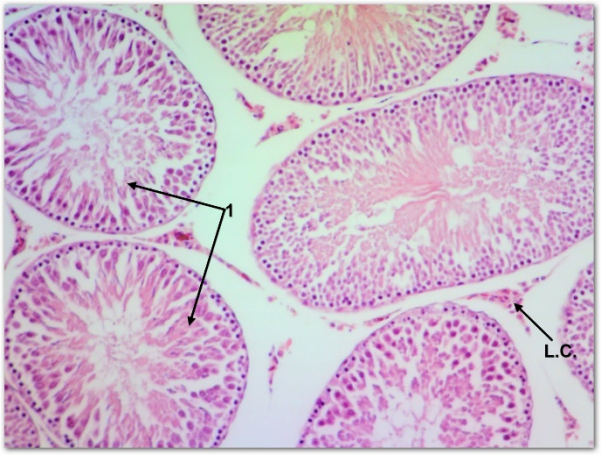


Fig. 15: A transverse section in rat testis from the four weeks $AlCl_3$+Royal jelly group shows tubules with hypoplasia and oligospermia (1) and normal leydig cells (L.C.).X100, H&E.

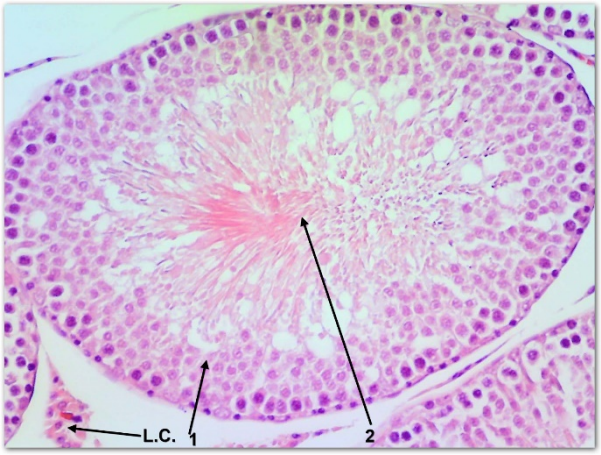


Fig. 16: A transverse section in rat testis from the four weeks $AlCl_3$+Royal jelly group shows tubule with normal epithelial cell lining (1), increase of sperm (2) and normal leydig cells (L.C.).X400, H&E.

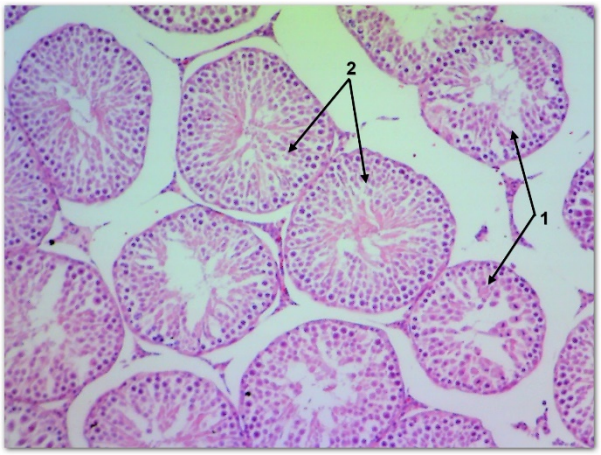


Fig. 17: A transverse section in rat testis from the six weeks $AlCl_3$+Royal jelly group shows tubules withhypoplasia and oligospermia (1) and tubule regainingtheir normal cell distribution (2). X100, H&E.

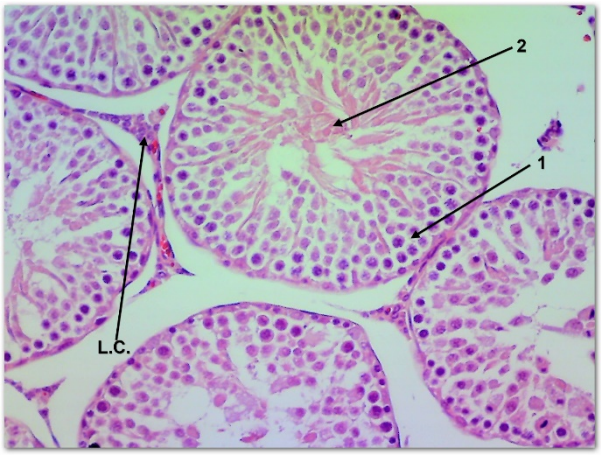


Fig. 18 : A transverse section in rat testis from the six weeks $AlCl_3$+Royal jelly group shows tubule with normal epithelialcelllining (1), increase of sperm (2) and normal leydigcells (L.C.). X400, H&E.

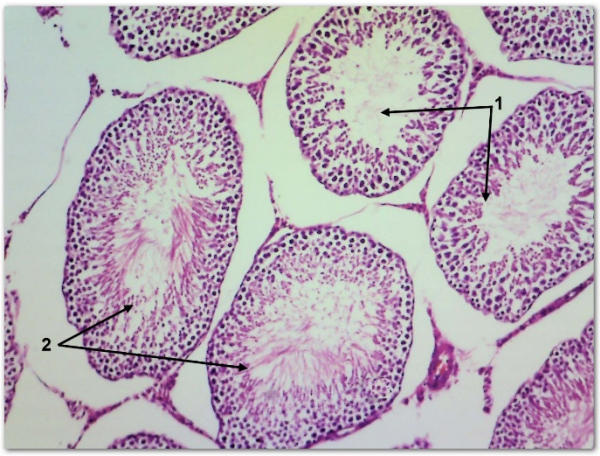


Fig. 19 : A transverse section in rat testis from the eightweeks $AlCl_3$+Royal jelly group shows tubules withhypoplasia and oligospermia (1) and tubule regainingtheir normal cell distribution (2). X100, H&E.

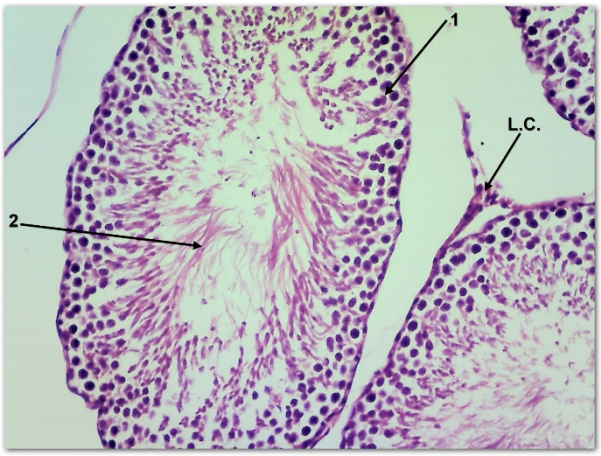


Fig. 20 : A transverse section in rat testis from the eightweeks $AlCl_3$+Royal jelly group shows tubule with normal epithelialcelllining (1), increase of sperm (2) and normal leydigcells (L.C.). X400, H&E.

DISCUSSION

Hormonal studies:

Table (1, 2 and 3) results showed a decrease in follicular stimulating hormone (FSH), luteinizing hormone (LH) and testosterone in the AlCl3 group compared to control. This is in agreement with [17] and [18] that said that AlCl3 significantly decreased plasma FSH, LH and testosterone. Reza and Palan, (2006) [17] & [19] found that calcium ion is important for FSH, LH and testosterone formation and secretion, Aluminum cross the blood brain barrier obstructed voltage-sensitive calcium cannels in cells that are responsible for gonadotropin releasing hormone (GnRH) formation and secretion. These diminished luteinizing hormone (LH) in the pituitary gland and then led to significantly lowered testosterone.

Results of this study showed a significant increase in FSH, LH and testosterone in royal jelly group and AlCl3+RJ group and therapeutic RJ group in most of experiment period. This observation is in agreement with the earlier findings of [20] who found that treatment with RJ significantly increased testosterone concentration in the RJ treated rats. Also, Najafi et al., 2014 [21] said that oxymetholone induce oxidative injury in mouse testis and showed a significant decrease in the serum testosterone concentration that was restored to near normal levels by RJ administration. Hassan, 2009 [22] demonstrated that RJ has a central effect as it contains acetylcholine. Acetylcholine is one of peripheral and central neurotransmitters [23]. However, acetylcholine helps to stimulate gonadotropin secretion of the hypothalamic level [24]. Therefore, RJ could increase LH level by its effect at level of hypothalamus via its content of acetylcholine. This elevation of LH level, is responsible for stimulation of testosterone secretion from interstitial cell [25]. Furthermore, testosterone could be elevated as a result of exogenous supplied by royal jelly, so it contains testosterone [22]. On the other hand, elevation of testosterone level could be attributed to zinc found in RJ. So zinc deficiency causes low testosterone level, while zinc supplementation can raise LH and testosterone level and increase fertility [26] & [27]. Royal jelly contains vitamin C, vitamin E, and it is rich in vitamin B6, B12 and that is the cause of its protective effect on sex hormones [22]. Mohamed et al., 2012 [28] resulted that plasma LH and testosterone levels were significantly decreased after Epinephrine injection in rats, while the administration of vitamin B complex following Epinephrine improved the plasma LH and testosterone levels. Vitamin B12 is needed to maintain fertility. In addition, vitamin B6 deficiency results in insufficiency of alteration of the functions of adrenal and pituitary glands, since it is involved in the synthesis of luteinizing hormone, estradiol and testosterone [29].

The current study showed a significant decrease in TSH, T4, T3 and T3/T4 of AlCl3 exposed group Table (4, 5, 6 and 7). Our results agree with [30] & [31] who said that AlCl3 significantly decreased TSH and thyroid hormones

T3 and T4. Utas et al., 2001 [32] found that TRH under normal physiological conditions, causes movement of intracellular calcium and induces influx of extracellular Ca2 + on thyrotropin cells. Presence of the calcium channel blocker could reduce calcium influx in vitro. Cannata-Andia and Fernandez-Martin, 2000 [33] suggested that TRH uses both intracellular calcium stores and extra cellular calcium through voltage-dependent calcium channels to raise intracellular calcium concentration causing TSH secretion. Release of TRH as a neurohormone depends on extracellular ca1cium [34]. Busselberg et al.,1993 [35] & Platt and [36] conclude that Aluminum is a powerful calcium channel b1ocker and inhibits both TRH and TSH release.

Thyroid hormone is a critical regulator of growth, development and metabolism in virtually all tissues, and altered thyroid status affects many organs and systems. A considerable amount of data shows that thyroid hormone influences steroidogenesis as well as spermatogenesis. The involvement of triiodothyronine (T3) in the control of Sertoli cell proliferation and functional maturation is widely accepted, as well as its role in postnatal Leydig cell differentiation and steroidogenesis. The presence of thyroid hormone receptors in testicular cells throughout development and in adulthood implies that T3 may act directly on these cells to bring about its effects [37]. In the present study aluminum chloride decreases thyroid hormones which affects testes tissue and that leads to low testosterone levels.

Royal jelly has an effect on thyroid function [38]. Treatment with RJ caused a significant increase in serum TSH, T4, T3 and T3/T4 in most weeks of experiment in RJ group and AlCl3+RJ group. That agrees with Rosoklija, (2012) [39] reported that RJ was recommended to patients for normalizing and maintaining the TSH normal level and normal size thyroid gland in hypothyroidism condition. And agrees with [40] who said that RJ is associated with an increase in TSH mRNA by 1.4-fold (indicative of synthesis of Thyroid Stimulating Hormone in the pituitary) and higher circulating T4 levels. Amit et al., 2012 [41] found that AlCl3 decreased vitamin B. Previous studies indicated that low concentrations of some B-vitamins may coexist with abnormal thyroid function in humans [42]. It was also found that there is an association between vitamin B12 deficiency and autoimmune thyroid disease [43]. In rats, vitamin B6 deficiency causes hypothyroidism of hypothalamic [44]. The activity of the hypothalamic-pituitary-thyroid axis in the setting of vitamin B6 deficiency has been studied in rats. Vitamin B6 deficiency leads to hypothyroidism resulting from decreased TRH synthesis in the hypothalamus. The reversal of vitamin B6 deficiency has led to normalization of thyroid hormone levels [44]. Mohamed et al.,2012 [28] resulted that administration of vitamin B complex following Epinephrine offered partial improvement in the levels of plasma level T3 and T4 hormones. RJ have high B vitamins content and that is the cause of its protective effect on thyroid stimulating hormone. Other studies showed that when RJ is added to the diet, most people notice an increased hormonal activity in terms of an improved sense of wellbeing and more energy. The regulation of RJ due to a big mount of vitamins in its contents [45] & [46].

Histological studies:

Results shows (Fig. 1 and 2) normal testicular structure in the control group. In the current study histological structures of the testes in the Aluminum chloride intoxicated rats showed the development of oligospermia, hypoplasia, congested blood vessels, exfoliated tubules, distorted tubule shape, lack of ordinary distribution of epithelial lining, expanded space between tubules with degeneration of interstitial tissue, and abnormal Leydig cells (Fig. 4, 5, 6, 7, 8, 9 and 10). Abdul-Rasoul et al., 2009 [47] found that these progressions may be attributed to impairment of sperm development and secretory functions of epididymal cells which might be due to oxidative stress or to inadequacy of androgens [48]. Mahran et al.,2011 [49] found histological changes as damages within the seminiferous tubules and vascular degeneration of the germ cells and Sertoli cells cytoplasm. The impairment caused by aluminum was accompanied primarily by the prolonged accumulation of aluminum in the mice testes. Khattab, 2008 [14] said that aluminum chloride caused testicular toxic as indicated by histological changes in the seminiferous tubules of the testes. That is because aluminum induced oxidative damage and the ability of aluminum to cross the blood-testis barrier after inducing oxidative stress and lipid peroxidation that damages the biological membrane of the testes and cause modification and atrophy of spermatogenic cells [18].

Aluminum chloride treatment elicited broad cytotoxic effects in the Leydig cells of mice. The presence of multiple lipid droplets in these cells could eventually lower secretory activity, probably by decreasing the use of free

cholesterol for steroidogenesis [50] & [51]. Two recent studies [52] & [53] have shown that intact mitochondria with active respiration are essential for LH-induced Leydig cell steroidogenesis.

The testis tissue structure of the RJ treated group (Fig. 11 and 12) was studied and showed no change from the control group, while the histological structures of testis of the AlCl3+Royal jelly group (Fig. 13-20) showed less tubules with hypoplasia and oligospermia and tubules with normal epithelial cell lining, increase of sperm and normal leydig cells.

El–Alfy et al., 2013 [54] observed the damage caused in the testes of mice after endoxan treatment displayed variable changes in both the seminiferous tubules and the interstitial tissue, while treatment with endoxan and RJ showed advanced observations. Hassan et al., 2012 [15] resulted that treatment of rats with RJ whose receiving cadmium chloride caused significant increase in the sperms count, the percentage of live sperms and the testis tissue concentration of ascorbic acid, associated with significant decreased in the abnormal sperms and testosterone hormone. Silici et al., 2009 [55] found that RJ ameliorated the cisplatin-induced reductions in weights of testes, epididymides, seminal vesicles, and prostate along with epididymal sperm concentration and motility. RJ contains spermatogenesis stimulating substances such as vitamin C, vitamin E, and arginine that protects from reproductive toxicity [22]. Moreover, it has been demonstrated that RJ inhibits the production of pro-inflammatory cytokines by activated macrophages [56]. Testosterone is essential for spermatogenesis from spermatogonium to spermatide [57]. RJ also contains L-arginine and carnitine amino acid, which essential for spermatogenesis [58]. Vitamin E and C is a well-documented antioxidant and has been shown to inhibit free-radical induced damage to sensitive cell membranes of the testis and reduced lipid peroxidation in tissue estimation by malodialdehyde, so vitamin E and C significantly decreased malodialdehyde, and increased in glutathione level [59].

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