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CanArabicgumimprovethe fertility impairment and ameliorate the oxidative stress induced by aluminumchloridein male rats?

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Abstract
Background*: Aluminum (Al) considered the third most ground element present everywhere which is nonessential and toxic metal to humans and accumulates in target organs causing serious effects on different systems including male reproductive system. Arabic gum (AG) is a dried exudate obtained from the stems and branches of Acacia Senegal tree and has antioxidant compounds that have ability to scavenging the free radicals, activation of antioxidant enzymes and inhibition of oxidases.**Aim****: This study evaluates the modulating effect of Arabic gum (AG)on fertility impairmentand oxidative stress-induced from exposure to aluminum chloride ($AlCl_3$) on adult male albino rats.**Materials and methods**:Thirty two male albino rats were divided randomly into four equal groups. Group I: served as control received only oral distilled water. Group II: rats were given AG by oral gavage (7.5 g/kg/day). Group III: received $AlCl_3$ (100 mg/kg) orally. Group IV: received oral $AlCl_3$ (100 mg/kg) and AG (7.5 g/kg) simultaneously for 60 consecutive days. Blood samples were taken to measure antioxidant state and testosterone level also semen samples were collected for semen analysisandhistopathological study was also done.*
Results*:Simultaneous administration of AG with $AlCl_3$ produced highly significant increase in plasma level of GSH, SOD and CAT with highly significant decrease in plasma level of MDA when compared to the $AlCl_3$ group. Fertility study regarding treatment of $AlCl_3$intoxicated rats with AG showed improvementin testosteronehormone level and semen analysis as there were highly significantincrease in testosterone hormone level, sperm count, sperm motility, sperm viabilitywith highly significant decrease in total sperm abnormality compared to $AlCl_3$ group. Also, histopathological changes occurred in $AlCl_3$ group were improved with concomitant administration of AG with $AlCl_3$.**Conclusion****: AG is an effective protective agent against $AlCl_3$-induced fertility impairment and oxidative stress due to its ability to decrease the oxidative stress and preserve the activity of antioxidant enzymes.*

Keywords:*Arabic gum, aluminum chloride, oxidative stress, fertility, testis.*

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I. Introduction

Aluminum (Al) considered the third most ground element which is nonessential and toxic metal in humans[1].With development in industries and increased pollution, aluminumgets more into our bodies through the air, water, foods and unfortunately drugs [2]. It can be found in food staff especially yellow cheese, salt, herbs, tea leaves and food additives [3].In addition, Aluminum salts such as aluminum chloride ($AlCl_3$) used in purification andtreatment of drinking water so it is easy entersour bodiesthroughGIT [4].Also aluminum used in homes, hospitals, schools, cosmetics as in deodorants [5].

Animal studies on aluminum toxicity revealed that aluminum accumulates in target organs and causeserious effects on various systems of the body including the male reproductive system[6].

Aluminumcausesits abnormalities through formation of (ROS) and decreasing of antioxidant system[7].

Also, aluminum causesits reproductive toxicity bydifferent mechanisms as increasing the oxidative stress, affecting spermatogenesis and damagingof the blood-testes barrier [6].

Necrosis of spermatocytes due to aluminum accumulation and notableimpairment in fertility also seen in both male rats and mice [8,9].

Intake of antioxidants created in the body or taken from food may improve the damage caused by oxidative stress by inhibiting of the oxidative chain reactions [10].

Arabic gum (AG) is a dried exudate obtained from the stems and branches of Acacia seyal and Acacia Senegal tree and rich in non-viscous soluble fibers [11].It is consists mainly of polysaccharides of high molecular weight, containing neutral and acids sugars [12], and minerals [13]. Gum Arabic has wide industrial uses in pharmacology

and foods [14]. The antioxidant characters of Gum Arabic were mentioned in many animal model systems by [15]. Gum Arabic as antioxidant contains flavone, catechin, polyphenols, tannins, chalcones, alkaloids and flavonoids [16]. These compounds have ability to scavenging the free radicals, activation of antioxidant enzymes and inhibition of oxidases [17].

Henceresearch on the effect of Arabic gum against aluminumchloride induced oxidative stress and fertility impairment has not so far been studied,therefore the present work aimed to evaluate this possible protective effect.

II. Materials and methods

Drugs:

1. Aluminum Chloride anhydrous ($AlCl_3$) was purchased from El-Gomhouria Co. For Trading Chemicals And Medical Appliances (Egypt).
2. Arabic gum(AG) was purchased in powder form with high purityfrom Sigma-Aldrich (St Louis, MO, USA).
3. All other chemicals were of the highest grade commercially available.

Animals:

Thirty two male albino rats, 5 months old, 200-250g used in this study obtained from Experimental Animal Breeding Farm, (Helwan-Cairo) for60 consecutive days. They were caged 8 per cage in well-ventilated place at room temperature at the department of pharmacology, Benha Faculty of Medicine. They allowed for 2 weeks of acclimatization with free water and standard food (pellets specific for rat feeding obtained from the animal breeding farm) on a schedule of 12 hours of light and 12 hours of dark. The time of doseadministration was fixed for all animals at 12 P.M. During the whole period of experiment, animals were treated humanely according to the protocol of handling of experimental animals of Benha Faculty of Medicine and every effort made to decrease the number of animals used and their suffering.

The animals were divided randomly into four equal Groups as following:

Group I: served as control receive only distilled water.

Group II: rats were given AG (7.5 g/kg/day)[18].

Group III: received $AlCl_3$ (100 mg/kg)[19].

Group IV: received $AlCl_3$ (100 mg/kg) and AG (7.5 g/kg) simultaneously.

The animals of all groups were given doses orally by nasogastric tube and the experiment remained for60consecutive days. At the end of the study,blood samples were collected in sterile tubes from all rats of different groups to measure antioxidant state and testosterone level evaluation. Then, animals were sacrificed by decapitation and dissected to obtain epididymis for semen analysis and histopathological study.

Antioxidant stateestimation

Plasma Glutathione (GSH) and Superoxide dismutase (SOD) concentrations were estimated spectrophotometric ally at 405 nm and 560 nm according to methods of**Beutler et al.** [20]and**Nishikimi etal.**[21], respectively.Malondialdehyde (MDA) was determined spectrophotometric ally in plasma at 534 nmaccording to**Onkawaet al.**[22]. Catalase (CAT) concentration was determined according to**Sinha**[23].

Testosterone hormone level and semen analysis assessment

Serum testosterone level was evaluated by radioimmunoassay according to**Wilson and Foster**[24]. Epididymistailcontent of each rat collected by squeezedslowly in sterileglass containing one ml of 2.9% sodium citrate solution for semen analysis according to**Bearden and Flaquary**[25].

Histopathological study

At the end of the study animals were sacrificed by decapitation and dissected then testes were removed and fixed in 10% buffered formalin then processed and stained with haematoxylin and eosin (H&E) according to **Banchroft et al.** [26],after that subjected to pathological examination by the light microscope.

Statistical analysis

Results were collected, tabulated and expressed as mean ± standard deviation.Statistical comparisons were performed using one-way analysis of variance (ANOVA) to compare mean values between treatment groups and control using SPSS version 16. P value ≤ 0.05 was considered statistically significant.

III. Results

Table (1): Statistical comparison between all studied groups regarding Malondialdehyde (MDA), plasma Glutathione (GSH), Superoxide dismutase (SOD) and Catalase (CAT) by ANOVA test

	Group (I) Control group	Group (II) Arabic Gum (AG) group	Group (III) Aluminum ($AlCl_3$) group	Group (IV) $AlCl_3$+ AG group	F test	P value
MDA level (umol/L)	10.42± 1.02	8.41±0.99 $**	28.42±1.23 $^**	13.60±0.98 $^€**	582	0.000**
GSH level (mmol/L)	72.26±1.80	79.72±0.99 $**	52.31±1.56 $^**	63.89±1.61 $^€**	477.76	0.000**
SOD level (u/ml)	296.90±7.26	309.70±1.15 $**	198.68±1.41 $^**	273.58±3.93 $^€**	110.3	0.000**
CAT level (u/ml)	20.38±1.31	24.53±1.25 $**	12.48±1.18 $^**	17.42±1.26 $^€**	131.55	0.000**

All values are expressed as mean±SD
Number of rats in each group = 8
The mean difference is significant at the 0.05 level.
highly significant(**)
$: indicate significant change as compared with a group (I)
^: indicate significant change as compared with a group (II)
€: indicate significant change as compared with a group (III)
F test= **ANOVA test**

The modulating effect of Arabic gum (AG) on the oxidative stress-induced byaluminum chloride ($AlCl_3$) exposure was obvious which is showed in **table (1)**. $AlCl_3$intoxicated groupresulted in highly significant decrease ($p<0.05$) in plasma level of GSH, SOD and CAT and highly significant increase ($p<0.05$) in plasma level of MDA when compared to the control group. Simultaneous administration of AG with $AlCl_3$ produced highly significant increase in plasma level of GSH, SOD and CAT with highly significant($p<0.05$) decrease in plasma level of MDA when compared to the $AlCl_3$ group. It is also notable that AG group showed highly significant($p<0.05$) increase in plasma level of GSH, SOD and CAT with highly significant ($p<0.05$) decrease in plasma level of MDA when compared to the control group.

Table (2): Statistical comparison between all studied groups regarding testosterone hormone level and semen analysis assessment by ANOVA test

	Group (I) Control group	Group (II) Arabic Gum (AG) group	Group (III) Aluminum ($AlCl_3$) group	Group (IV) $AlCl_3$+ AG group	F test	P value
Testosterone hormone level (ng/ml)	2.54±0.68	3.47±0.85 $**	1.38±0.72 $ ^**	2.39±0.68 ^€**	10.84	0.000**
Sperm count ($x10^6$/ml)	2.33±0.49	2.83±0.23 $**	1.61±0.48 $ ^**	2.47±0.53 ^ €**	8.08	0.000**
Sperm motility (%)	85.64±3.71	91.52±2.33 $**	72.34±1.36 $^**	83.17±0.81 $^€**	94.88	0.000**
Sperm viability (%)	90.36±1.17	94.22±0.90 $**	79±0.7 $^**	84.16±0.73 $^€**	451.21	0.000**
Total abnormality (%)	10.08±0.65	7.13±0.66 $**	33.05±0.66 $^**	12.09±0.63 $^€**	2.63	0.000**

All values are expressed as mean±SD
Number of rats in each group = 8
The mean difference is significant at the 0.05 level.
highly significant(**)
$: indicate significant change as compared with a group (I)
^: indicate significant change as compared with a group (II)
€: indicate significant change as compared with a group (III)
F test= **ANOVA test**

Regarding testosterone hormone level and semen analysis**table (2)** showed that $AlCl_3$intoxication resulted in highly significant ($p<0.05$) decrease in testosterone hormone level, sperm count, sperm motility, sperm viability with highly significant increase ($p<0.05$) in total sperm abnormality when compared with the control group. On the other hand, when AG was simultaneously administrated with $AlCl_3$ there was highly significant increase ($p<0.05$) in testosterone hormone level, sperm count, sperm motility, sperm viability with

highly significant ($p<0.05$) decrease in total sperm abnormality when compared with $AlCl_3$ group. Also it is notable that AG group showed highly significant ($p<0.05$) increase intestosterone hormone level, sperm count, sperm motility, sperm viability with highly significant ($p<0.05$) decrease in total sperm abnormality when compared with the control group.

Concerning the histopathological study, the testis of control group showed normal architecture with crowded, closely packed seminiferous tubules lined by stratified spermatogenicepithelium with spermatozoa seen within the lumen andthe interstitial spaces show Leydig cells **(Fig.1)**.

The testis of AG group (group II)showed normal architecture with crowded, closely packed seminiferous tubules with spermatozoa seen within the lumen and the interstitial spaces show Leydig cells **(Fig.2)**.

The testis of $AlCl_3$ group (group III) showed degenerated spermatogenic epithelium and loss of the releasing spermatozoa. Also pyknotic nuclei of the spermatogenic cell were seen; the interstitial spaces show eosinophilic fluid (edema) with infiltration of mononuclear cells and vacuolation**(Fig.3)**.

The testis of $AlCl_3$+ AG group (group IV) showed normal shaped seminiferous tubules lined by stratified spermatogenic epithelium with normal releasing spermatozoa within the lumenandthe interstitial space shows accumulation of eosinophilic fluid (edema) **(Fig.4)**.

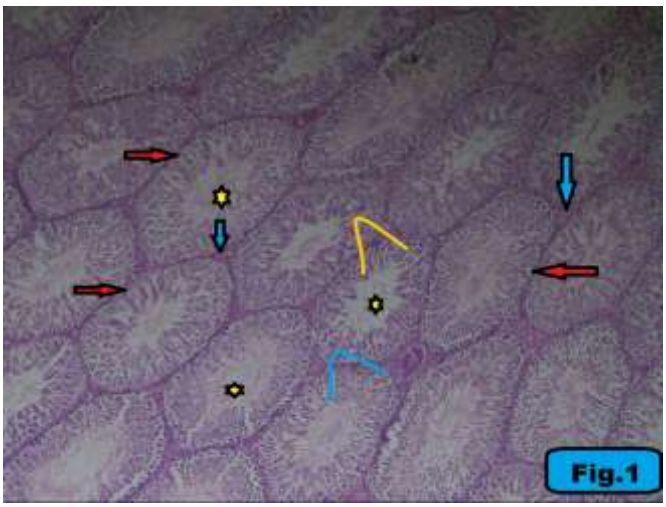

Fig.(1): Photomicrograph section in the testis of control group shows normal architecture with crowded, closely packed seminiferous tubules (red arrow) lined by stratified spermatogenic epithelium (orange and blue lines) with spermatozoa (yellow star) seen within the lumen. The interstitial spaces show Leydig cells (blue arrow)**(H & E X40)**.

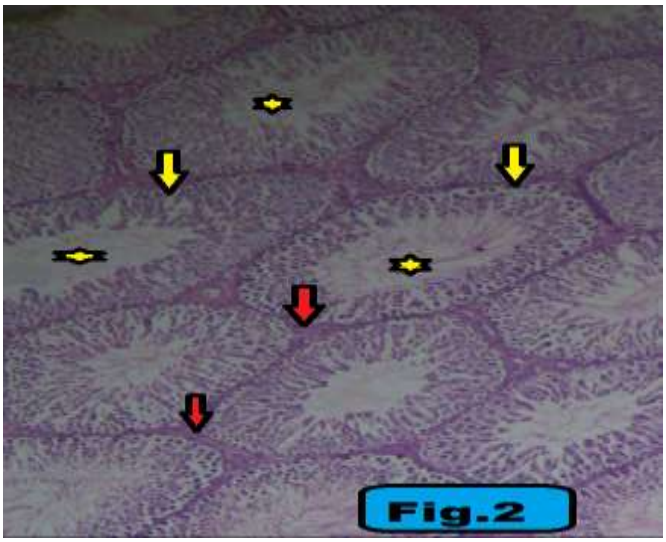

Fig.(2): Photomicrograph section in the testis of Arabic Gum group (group II) shows normal architecture with crowded, closely packed seminiferous tubules (yellow arrow) with spermatozoa (yellow star) seen within the lumen. The interstitial spaces show Leydig cells (red arrow)**(H & E X40)**.

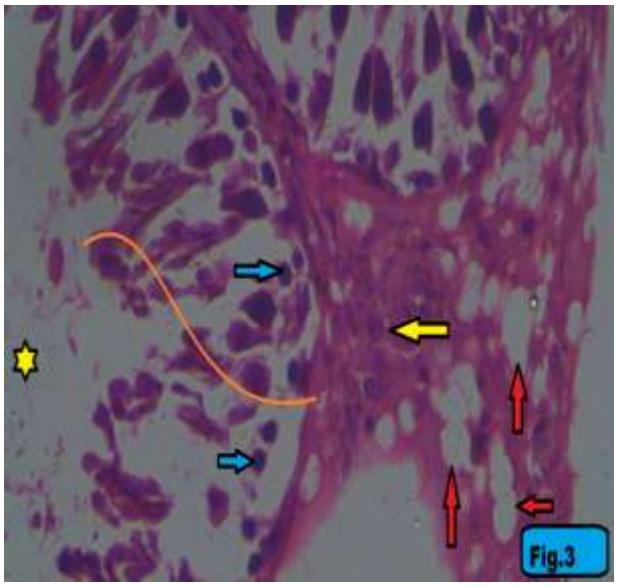

Fig. (3): Photomicrograph section in the testis of aluminium chloride group (group III) shows degenerated spermatogenic epithelium (orange line) and loss of the releasing spermatozoa (yellow star). Pyknotic nuclei of the spermatogenic cell (blue arrow). The interstitial spaces show eosinophilic fluid (edema) with infiltration of mononuclear cells (yellow arrow) and vacuolation (red arrow) **(H & E X400)**.

Fig. (4): Photomicrograph section in the testis of aluminium chloride+ Arabic Gum group (group IV) showsnormal shaped seminiferous tubules lined by stratified spermatogenic epithelium with normal releasing spermatozoa (red star) within the lumen. The interstitial space shows accumulation of eosinophilic vacuolated fluid (yellow star) **(H & E X40)**.

IV. Discussion

The widespreadof aluminum in the surrounding environment makes the exposure to this pollutant inevitable[27]. These days, excess exposure to aluminum by different way increases the risk of its toxicity which results in hazardous effect to different body systems[28].

Gum Arabic as antioxidant contains flavone, catechin, polyphenols, tannins, alkaloids and flavonoids [16]. These compounds have ability to scavenging the free radicals, activation of antioxidant enzymes and inhibition of oxidases [17].

The currentstudy aimed to evaluate the protective effect of Arabic gum (AG) against the oxidative stress and fertility impairment resulted from exposure to aluminum chloride ($AlCl_3$) intoxication.

Concerning the effect of $AlCl_3$ on oxidative stress, the currentwork revealed that $AlCl_3$ intoxication resulted in highlysignificant decrease in plasma level of GSH, SOD and CAT with highlysignificant increase in plasma level of MDA.This is similar to the results reported by **Abdelazem**[29]who revealed that there was decrease in GSH, SOD and CAT activity with increase in MDA activity in aluminum intoxicated rats.Also **Hammoud and Shalaby**[30]founded in their study on rats that $AlCl_3$ resulted in decrease in plasma level of GSH and SOD and increase in plasma level of MDA. Also this result was in agreement with the study done by **Cheraghiet al.** [31]who reported that aluminum resulted in oxidative stress in rat's testes and cause changes in the antioxidant system. This may be resulted from ability of aluminum to produce oxidative stress, cross the blood-testes barrier and activation of lipid peroxidation system[9].

Also in the current work simultaneous administration of AG with $AlCl_3$ produced significant increase in plasma level of GSH, SOD and CAT with significant decrease in plasma level of MDA compared to $AlCl_3$ group. Also, AG group showed highly significant increase in plasma level of GSH, SOD and CAT with highly significantdecrease in plasma level of MDA compared to the control group.

This could be attributed to the excellent antioxidant properties of AG;these properties seem to be due to its ability to scavenge free radicals[32].AG is also rich in antioxidants which reported to have strong reducing effect on lipid peroxidation [17].

AG reported to have antioxidant effect which improve lipid metabolism in experimental animal studies[33]. Such results were previously provided by **Ahmed et al.** [34] who stated that AG may improve the hepatic expression of oxidative stress genes hence improve antioxidant status.

Kaddam et al. [35]founded that AG has a potent antioxidant effect on sickle cell anemia and other diseases characterized by oxidative stress. Also **Kamal et al.** [36] studied the effect of AG and founded that AG has a favorable immune modulator effect on rheumatoid arthritis.

Also**Gamal El-din et al**. [37]reported that AG has anti-inflammatory effect and used internally and externally for the treatment of inflammation.

As regarding fertility profile, the current study revealed that $AlCl_3$resulted in highly significant decrease in testosterone hormone level, sperm count, sperm motility, sperm viability with highly significant increase in total sperm abnormality.

Pandey and Jain[38]reported that aluminum may be responsible for decrease in sperm motility, viable sperms and causes morphological abnormalities.

Also **AL Dera and Abushouk**[39] **and Pandey and Jain** [38]postulated that aluminum impairs the male fertility and disturbs spermatogenesis by oxidative stress mechanism and decrease in the serum testosterone, follicle stimulating hormone and luteinizing hormone.**Guo et al.** [9] **and Yousef et al.** [40]founded that high levels of aluminum in spermatozoa and seminal plasma of humans resulted in decrease in sperm viability and motility.

As regarding the histopathological study, it caused degeneration in spermatogenic epithelium and loss of the releasing spermatozoa. Also pyknotic nuclei of the spermatogenic cell were seen; the interstitial spaces show eosinophilic fluid (edema) with infiltration of mononuclear cells and vacuolation.

Hammoud and Shalaby[30]stated thataluminum intoxication resulted in degeneration of spermatogoneal cells lining seminiferous tubules and incomplete spermatogenesis.

V. Conclusion

From the results of the current study it can be concluded that Arabic gum is an effective protective agent against aluminum chloride-induced fertility impairment and oxidative stress due to its ability to decrease the oxidative stress and preserve the activity of antioxidant enzymes.

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