A Histopathologic Study of the Protective Effect of Grape Seed Extract Against Experimental Aluminum Toxicosis in Male Rat

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Abstract:
Grape seed extract is considered as a concentrated source of polyphenols, more specifically polyhydroxylated flavan-3-ols. It is increasingly receiving attention as dietary supplement for the homeostatic management of inflammation, to support detoxification, and for anticancer, antioxidant, weight loss, and other benefits. The present pathologic study was carried out to investigate the microscopic changes related to the protective effect of supplementation with grape seed extract (GSE) against the induced lesions due to experimental intoxication with aluminum chloride ($\text{AlCl}_3$) as one of the common environmental pollutants. For this aim, 4 groups of male rats (-ve control, +ve control for GSE supplementation, +ve control for $\text{AlCl}_3$ intoxication, and experimental group of concurrent administration of GSE and $\text{AlCl}_3$), were used. At the end of the experiment (8 weeks) the rats of all groups were necropsied and tissues specimens were collected and processed for the following comparative histopathologic examination. The obtained microscopic findings, revealed an alleviating effect of GSE against $\text{AlCl}_3$-induced lesions in various body organs of the rats.

Key Words: Grape seed extract; Aluminum; Aluminum chloride; oxidants; Antioxidant; neurotoxicosis; hepatotoxicosis; nephrotoxicosis; protective effect.

Abbreviations: GSE= Grape seed extract; Al=Aluminum; AlCl$_3$= Aluminum chloride

Introduction:
Recently consumption of antioxidants has increased day after day. Plant-derived polyphenols are increasingly receiving attention as dietary supplements of antioxidants for the homeostatic management of inflammation, support detoxification, anticancer, weight loss, and other benefits (Parris., 2009). Grape seed extract (GSE) was reported to have many other beneficial effects like the hepatoprotection (Akram, et al., 2008), protection for silica-induced pulmonary fibrosis (Ali, et al., 2008) and ethanol-induced cell death (Wen-Hsiung, and Ying-Jing, 2006), as well
as antioxidant properties and free radical scavenging activities (Fauconneau, 1998). The complex grape seed proanthocyanidin mix (including catechin and epicatechin monomers and oligomers) was reported to counter the oxidative stress, protects the circulatory system, and has anti-inflammatory and anticancer effects (Katiyar, 2008; Sharma et al., 2007; and Natella et al., 2002). Grape seed extract is also known to be a concentrated source of polyphenols, more specifically polyhydroxylated flavan-3-ols. The grape seed polyphenols resemble the catechins of green tea in basic molecular structure (flavan-3-ols and their gallates), except grape seed constituents (Vigna et al., 2003; Aldini et al., 2003 and Donovan et al., 2002).

Aluminum (Al) is known to be a highly neurotoxic element and was suggested to play a role in degeneration of nerve cells in brain of the experimental animals (Yumoto et al., 2000). Chronic exposure to Aluminum can also cause other alterations in skeletal, hemopoietic and respiratory systems rather than the nervous system (Chen et al., 2002). Sahar Abd El-Rahman (2003) described the pathologic defects due to aluminum toxicity in rats to be included spongioform changes in the neurons specially those of hippocampus, nuclear deformity, and neurofibrillary degeneration similar to neurofibrillary tangles in Alzheimer’s disease. Amarpreet and Kiran (2005) mentioned that the toxic effects of chronic aluminum intoxication could be mediated through modifications in the intracellular calcium homeostasis, which may lead to impaired neuronal function.

Few studies were carried out concerning the protective trials against Aluminum toxicosis in animals. The in vitro studies of Khanna and Nehru (2007) reported that the glial cells and neurons isolated from rat cerebral cortex are more capable of handling the oxidative stress due to the exposure to Aluminum at the dose level of 100 mg/kg b.w. for 8 weeks. Kutlubay et al. (2007) reported that the different forms of Aluminium (Al) are environmental xenobiotics that induce free radical-mediated cytotoxicity and reproductive toxicity. Several studies were carried out in order to elucidate the protective effects of vitamin E towards aluminium toxicity on the histology of the rat testis. In conclusion, they found that vitamin E antagonizes the toxic effects of Al at the histological level, thus potentially contributing to an amelioration of the testis histology in the Al-treated rats. Albendea et al. (2007) reported that melatonin confers protection against Al-induced oxidative damage in synaptosomes and suggest that this indoleamine may be considered as a neuroprotective agent in Al toxicity because of its antioxidant activity. L-ascorbic acid was found to be effective
in alleviating the toxicity of aluminium chloride (AlCl₃) on certain hematobiochemical parameters, lipid peroxidation and enzyme activities of male New Zealand white rabbits (Yousef, 2004). The present study aimed to provide a histopathologic evaluation for the protective effect of GSE, against the oxidative damage-induced by aluminum intoxication in tissues of male rats.

**Materials and Methods:**

**Materials:**

1. Laboratory animals: Eighty adult male Sprague-Dawley rats (of about 2 months old and of 150-200 g body weight), were obtained from Animal House of Veterinary College and Animal Resources, King Faisal University. The rats were put for about one week of acclimatization at standard hygienic conditions, fed on standard rat feed (Table 1) and water ad libitum.

| Table (1) |
| Ingredients of the used standard rat diet and their percentage or amounts in the diet. |

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Percentage</th>
<th>Ingredients</th>
<th>Percentage</th>
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<tbody>
<tr>
<td>Crude protein</td>
<td>18 %</td>
<td>Vitamin A</td>
<td>15.5 IU/g</td>
</tr>
<tr>
<td>Crude oil</td>
<td>05 %</td>
<td>Vitamin E</td>
<td>101 mg/kg</td>
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<tr>
<td>Carbohydrate</td>
<td>57 %</td>
<td>Vitamin K</td>
<td>51 mg/kg</td>
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<tr>
<td>Sugar</td>
<td>05 %</td>
<td>Vitamin B1</td>
<td>17 mg/kg</td>
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<tr>
<td>Starch</td>
<td>20 %</td>
<td>Vitamin B2</td>
<td>15 mg/kg</td>
</tr>
<tr>
<td>Niacin</td>
<td>41 mg/kg</td>
<td>Vitamin B6</td>
<td>18 mg/kg</td>
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<tr>
<td>Biotin</td>
<td>0.4 mg/kg</td>
<td>Vitamin B12</td>
<td>0.1 mg/kg</td>
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<tr>
<td>Foliate</td>
<td>3.5 mg/kg</td>
<td>Vitamin D3</td>
<td>1.5 IU/g</td>
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2. Chemicals: The used Grape seed extract (GSE) was obtained from Traco Labs (Champaign, IL), USA, while the (AlCl₃) was obtained from Sigma. The GSE-standardized preparation, contains 89.3% (wt/wt) procyanidins, 6.6% of monomeric flavonols, 2.24% of moisture content, 1.06% of protein, and 0.8% of ash.
Methods:

1. Experimental design: The used rats were randomly subdivided into 4 groups (each of 20 rats), caged in a separate metal cages and received the following treatments:
   - Group 1 (= - ve control): Received drinking water (free from AlCl₃) ad libitum, and fed on the standard rat feed all over the 8 weeks of the experiment.
   - Group 2 (= + ve control for GSE): Daily fed on 0.5 % GSE diet and drink water (free from AlCl₃) ad libitum, all over the 8 weeks of the experiment.
   - Group 3 (= + ve control for AlCl₃ intoxication): Fed on the standard rat feed and received daily oral doses of 37 mg/ kg.b.w. AlCl₃ (1/10 LD50 = 370 mg / kg.b.w., according to Scott, 2000), in the drinking water, all over the 8 weeks of the experiment.
   - Group 4 (= experimental group for concurrent administration of GSE with AlCl₃): Daily fed on 0.5 % GSE diet and drink water free from AlCl₃, for one week; followed by the concurrent daily fed on 0.5 % GSE diet with drink water contained AlCl₃), till the end of the experiment.

The rats of the different groups were observed for recording any clinical signs or mortalities. At the end of 8 weeks of the experiment the rats of all groups were euthanized and subjected for necropsy. Tissue specimens were collected from different body organs, for histopathologic studies.

2. Histopathologic techniques: The collected tissue specimens from all animal groups were fixed in 10% neutral buffered formalin solution then passed automatically through the routine paraffin embedding technique, sectioned at 5 microns and then stained with Hematoxylin and eosin stain (Drury and Wallington, 1980) for histopathologic examination.

Results:

Animal in different groups, with exception for the 3rd group of intoxication with AlCl₃ has not exhibited any abnormal clinical signs and were apparently healthy all over the 8 weeks of the experiment. The intoxicated rats of this 3rd group (The +ve control for AlCl₃ toxicosis) exhibited some mild nervous signs of restlessness, fairness with some excitations, especially after 6 weeks of intoxication. The necropsy findings in these rats revealed mild to moderate degrees of congestion in various body organs, especially in the brain tissue.
The microscopic examination of the examined organs in rats of the 1st (-ve control) and 2nd (+ve control for supplement with GSE) groups revealed normal histologic criteria. The detected microscopic alterations in case of the 3rd group of intoxication with AlCl₃ were obviously seen in the brain, testes, epididymis, prostate glands, kidneys, liver, lungs and spleen. The cerebral and cerebellar meninges showed severely congested blood vessels (Fig. 1), while the deep cerebral cortex suffered variable degrees of neuronal degeneration, satellitosis and neuronophagia (Fig. 2). The blood vessels at the mid brain appeared also dilated and congested and in some areas associated with perivascular cuffing with lymphocytes (Fig. 3). The testis in these cases showed some changes of vacuolar degeneration in the seminiferous tubules in addition to interstitial edema with congested blood capillary (Fig. 4). In some other areas the seminiferous tubules appeared with variable degrees of degeneration, necrosis and severe damage (Figs. 5 and 6). The stored spermatozoal contents in the lumina of the epididymal tubules, especially at the tail region contained some numbers of the large nucleated immature spermatocytic cells (Fig. 7). The secretory acini of the prostate gland appeared active with cystic dilated and in some areas appeared widely separated with an interstitial edema and congested blood vessels (Fig. 8).

The kidney showed also changes of vacuolar degeneration of the epithelium in some renal tubules in addition to cast formations as well as intertubular infiltration with mononuclear cells (Fig. 9). The changes in liver were manifested by hepatocytic degenerations and necrosis in addition to severe vascular congestion, especially in the portal blood vessel (Fig. 10). The microscopy of the lungs in these cases of intoxication revealed excess numbers of the bronchiolar, peribronchiolar and alveolar contents of mononuclear cells (Fig. 11). Spleen appeared suffered dispersion with depletion of the lymphocytic elements of the follicles (Fig. 12).

The microscopic examination for the organs in rats of the 4th group of concurrent administration of GSE and AlCl₃ revealed the followings: Brain lesions of only mild vascular congestion, especially at the deep cerebral cortex (Fig. 13). The testis appeared with nearly normal seminiferous tubules and interstitial elements (Fig. 14). The epididymis of these cases appeared also with nearly normal tubules that contained an excess of the normally stored spermatozoa contents (Fig. 15). The secretory acini of the prostate gland in these cases appeared also normal with folded secretory epithelium (Fig. 16). The kidneys of these treated rats showed nearly normal glomeruli and renal tubules (Fig. 17). The hepatic lobules in the livers
appeared also with nearly normal hepatocytic elements (Fig. 18). The alveoli in the lungs of these treated rats appeared with some variable numbers of the neutrophilic elements (Fig. 19), while the spleen of these cases appeared nearly normal with well developed lymph follicle thick walled central arteriole (Fig. 20).

**Discussion:**

Large amounts of Al are used in daily life; it is contained in many sources as food additives, toothpastes, deodorants and anti-acids. It has been proposed that Alzheimer disease may be caused by irreversible accumulation of Al in the brain, especially in the brain cell nuclei (Yumoto et al., 2000). The present work was done in order to investigate the suggested protective effect of supplementation with GSE, as a powerful antioxidant against the known toxic effects of AlCl$_3$, in the organs of the experimentally intoxicated male rats. The experiment was extended for 8 weeks. The observed clinical signs of intoxication were seen only in the rats of the +ve control group for AlCl$_3$ intoxication. These signs were in the form of mild to moderate nervous manifestations of restlessness, fairness with some excitations, especially after 6 weeks of the daily oral administration for AlCl$_3$. The observed necropsy findings in these rats were restricted on the mild to moderate degrees of congestion in various body organs, especially in the brain tissue in addition to patches of grayish-white discoloration in the lungs.

The present microscopic examination for the examined organs of rats in the -ve control as well as +ve control group for GSE, revealed normal histologic criteria of the tissues. On the other hand the examined organs in the +ve control group for Al intoxication, many lesions of toxicosis and oxidative stress were seen. These findings were agree with Zatta, et al., (2002). These findings of toxicosis in the brain tissue were in the form of vascular congestion, perivascular cuff with lymphocytes, neuronal degeneration, necrosis and neuronophagia. These changes of neurotoxicosis are explained to be related to the accumulation of Al together with the fact that glial cells are particularly vulnerable to Al exposure (Struys-Ponsar et al., 1997; Suarez-Fernandez, 1999; and Fattoretti, et al., 2003).

The present lesions of Al toxicosis in the testes, epididymis and prostate glands were in the form of vascular congestion, edema, vacuolar degeneration in some of the seminiferous tubules and necrosis or complete damage in other tubules, epididymal contents of some large and nucleated immature spermatocytic cells mixed with the other normal spermatozoal contents and cystic dilatation of the prostatic acini with interstitial edema.
and vascular congestion. These findings are in agreement with Kutlubay et al., (2007). The present lesions of Al nephrotoxicosis in the kidneys were commonly consisted of tubular changes of epithelial vacuolar degeneration and cast formations in addition to intertubular mononuclear cell infiltrations. These findings are in agreement with Mahieu, et al., (2009). The changes of hepatotoxicosis in the present work were also manifested by the hepatocytic degenerations and necrosis besides severe congestion of the portal blood vessels. These are similar to the findings of Bogdanovi, et al., (2008). The present lesions of toxicosis in the lungs of the present work were not restricted on the vascular congestion but were associated with numerous areas of bronchopneumonia characterized by an excess of bronchiolar, peribronchiolar and alveolar infiltrations of mononuclear cells (Senlin, et al., 2009). Spleen in the present work appeared with changes of Al toxicosis in form of depletion of the lymphocytes with dispersion of the lymph follicles.

The present findings for microscopic examination of (brain tissue, male genital organs, kidneys, liver, lungs and spleen) in case of concurrent administration of the GSE with AlCl$_3$ revealed less or no obvious lesions of Al-toxicosis with less exception in the lungs. The lungs appeared with less or delayed protective effect where the pulmonary alveoli still in the stage of resolution and appeared to contained some mononuclear cells. In the brain tissue only mild vascular changes of congestion with very less or no changes of neuronal degeneration, were seen. These findings explain the occurrence of an alleviating histological effect in comparison to the detected lesions of neurotoxicosis in the +ve control group for aluminum intoxication. Similar results were obtained in other studies for the ameliorating effects of the antioxidants on Al-toxicity (Yousef, 2004; Albendea et al., 2007; and Khanna and Nehru, 2007).

The microscopic examination for the testes, epididymis and prostate glands, in cases of the present concurrent administration of GSE and AlCl$_3$ revealed also nearly normal histologic criteria. These findings for improvements in the genital organs are in agreement with the finding of Kutlubay et al., (2007) for the protective effect of Vitamin E towards Al-toxicity in rat. Similarly the present findings of nearly normal microscopic findings in the kidneys, liver and spleen are supporting the findings for the protective effect for GSE on Al-toxicosis (Punita and Dhawan, 2009).

In conclusion for all of the present findings, the supplementation with the GSE resulted in an appreciable improvement in the architecture as well as protective effect for Al toxicosis in body organs with less exception for
the lungs which may need either higher doses of GSE supplementation or prolonged time of administration. Therefore this study shows that GSE has a potential to exhibit protective role in the condition of Al-induced oxidative stress and be explored further to be considered as an additional promising drug against Al-toxicosis.

**Acknowledgements:**
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Legend of figures:

Fig.1: Cerebellar meninges of intoxicated rat with AlCl₃: severely congested meningeal blood vessels. H and E. X 400.

Fig.2: Deep cerebral cortex of intoxicated rat with AlCl₃: neuronal degeneration, satellitosis and neuronophagia. H and E. X 400.

Fig.3: Mid brain of intoxicated rat with AlCl₃: Congestion with excess perivascular cuffing with lymphocytes. H and E. X 400.

Fig.4: Testis of intoxicated rat with AlCl₃: Degenerated seminiferous tubule (Right), interstitial edema with congested blood capillary. H and E. X 400.
Fig.5: Testis of intoxicated rat with AlCl₃: Degenerated seminiferous tubule (Right) and other necrotic seminiferous tubule (Left). H and E. X 400.

Fig.6: Testis of intoxicated rat with AlCl₃: Completely damaged seminiferous tubule (Left). H and E. X 400.

Fig.7: Epididymis of intoxicated rat with AlCl₃: Numerous large nucleated immature spermatocytic cells in the lumina. H and E. X 400.

Fig.8: Prostate gland of intoxicated rat with AlCl₃: Cystic acini with interstitial edema and congested blood vessels. H and E. X 400.
Fig.9: Kidney of intoxicated rat with AlCl$_3$: Vacuolar degenerated epithelium of some renal tubules with cast and intertubular infiltration with mononuclear cells. H and E. X 400.

Fig.10: Liver of intoxicated rat with AlCl$_3$: Hepatocytic degeneration and necrosis with severely congested portal blood vessel. H and E. X 400.

Fig.11: Lung of intoxicated rat with AlCl$_3$: Bronchiolar, peribronchiolar and alveolar contents of mononuclear cells. H and E. X 400.

Fig.12: Spleen of intoxicated rat with AlCl$_3$: Dispersion with depletion of the lymphocytic elements. H and E. X 400.
Fig.13: Deep cerebral cortex of rat after concurrent administration of GSE with AlCl₃ intoxication: congested deep blood capillary. H and E. X 400

Fig.14: Testis of rat after concurrent administration of GSE with AlCl₃ intoxication: Nearly normal seminiferous tubules and interstitial elements. H and E. X 400.

Fig.15: Epididymis of rat after concurrent administration of GSE with AlCl₃ intoxication: Nearly normal tubules with excess of the normally stored spermatozoa contents. H and E. X 400.

Fig.16: Prpstate gland of rat after concurrent administration of GSE with AlCl₃ intoxication: Nearly normal secretory acini with folded epithelium. H and E. X 400.
Fig. 17: Kidney of rat after concurrent administration of GSE with AlCl\textsubscript{3} intoxication: Nearly normal glomeruli and renal tubules. Congestion of one of the deep cerebral blood capillaries (arrow). H and E. X 400

Fig. 18: Liver of rat after concurrent administration of GSE with AlCl\textsubscript{3} intoxication: Hepatic lobule with nearly normal hepatocytes. H and E. X 400.

Fig. 19: Lung of rat after concurrent administration of GSE with AlCl\textsubscript{3} intoxication: Alveoli with some variable numbers of the neutrophilic elements. H and E. X 400.

Fig. 20: Spleen of rat after concurrent administration of GSE with AlCl\textsubscript{3} intoxication: Nearly normal well developed lymph follicle with thick walled central arteriole. H and E. X 400.
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دراسة نسيجية مرضية للتأثير الواقعي لمستخلص بذر العنب
ضد التغييرات التسممية التجريبية للألومونيوم في ذكور الجرذان

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الملخص:
إن التلوث البيئي (اللواء والمواد الغذائية) بالعديد من الكيمياء والعناصر الأخرى والغير ضرورية مثل المعادن الثقيلة يجعل من مساوئ ومخلوفات الصناعات والتنقيبات الحديثة والድقة. وينبغي التلوثات بذور النباتات التي تؤثر على الصحة والصحة، وما يتضمن عليه أعضاء الجسم للإنسان والحيوان من أهم مصادر الخطر على الصحة وما يتضمن عليه أعضاء الجسم للإنسان والحيوان من أهم مصادر الخطر.

إن الآثار البيئية (اللواء والمواد الغذائية) بالعديد من الكيمياء والعناصر الأخرى والغير ضرورية مثل المعادن الثقيلة يجعل من مساوئ ومخلوفات الصناعات والتنقيبات الحديثة والדים. وينبغي التلوثات بذور النباتات التي تؤثر على الصحة والصحة، وما يتضمن عليه أعضاء الجسم للإنسان والحيوان من أهم مصادر الخطر.

ونظراً لقلة عدد المنشورات حول صحة تجريبي للاستقلال على مدى تأثير مستخلص بذر العنب على تلك الآثار التسممية للكبريتيد الألومونيوم على أعضاء الجسم تجريبياً في ذكور الجرذان. من أجل هذا الدفعة تم تقسيم الحيوانات عشوائياً إلى أربعة مجموعات: مجموعة ضابطة سلبية (لم تعطى أي معاملات)، مجموعة ضابطة إيجابية لجرعات مستخلص بذر العنب فقط، مجموعة ضابطة إيجابية للجرعات السامة للكبريتيد الألومونيوم، ومجموعة أخرى وهي هدف الدراسة. فقد تم معاملتها بجرعات يومية من مستخلص بذر العنب لمدة أسبوع قبل التعامل المزدوج مع الجرعات السامة للكبريتيد الألومونيوم يومياً طوال مدة التجربة والتي استغرقت ثمانية أسابيع. وقد تم تركيز الدراسة على التحليل المجهرى للأعضاء. ولقد خلصت هذه الدراسة إلى أن مستخلص بذر العنب له دور وقائي وإيجابي
على غالبية أنسجة أعضاء الجسم المختلفة (المخ، الأعضاء التناسلية الذكرية، الكبد، الكلى والطحال) ضد تأثير الإجهاد التأكسدي الناتج عن مكلوريد الألومونيوم وبالتالي فإنه يمكن أن ينصح به كمصدر الإضافات أو العناصر ضد التسمم بالألومونيوم.