# Possible Role of $\gamma$ -Irradiated Ginseng in the Modulation of Some Biochemical Disorders Produced by Aluminum in Rats

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> LUMINUM (Al) toxicity in human and animals has been Aa matter of concern. Ginseng is liable to be contaminated by micro-organisms during the pre- and post-harvest handlings. Thus, ginseng decontamination by  $\gamma$ - rays is needed to warrant the microbiological quality. The aim of this study is to demonstrate the protective effect of y-irradiated ginseng against Al-induced toxicity in rat model. Male albino rats were divided into four groups of 8 rats : A control group, the yirradiated ginseng extract (IGE) group; daily received the IGE (400 mg kg<sup>-1</sup> body wt) for 6 weeks, the Al group received orally a daily dose (0.5 mg kg<sup>-1</sup> body wt) of aluminum chloride (AlCl<sub>3</sub>) for 6 weeks and the irradiated ginseng extract+ AlCl<sub>3</sub> (IGE-Al) group, received IGE+ AlCl<sub>3</sub> for 6 weeks. Al administration significantly decreased some haematological parameters, sex hormones. Antioxidant enzymes activity in liver and testes showed a decrease. The results also showed a significant increase in some hepatic marker enzymes associated with an elevation of tissue lipid peroxidation (LPO). When experimental animals received IGE+ ALCL<sub>3</sub>, all these parameters were restored to approximately the control levels. These results demonstrated that administration of  $\gamma$ -irradiated ginseng could be effective in the protection against the toxicity of AlCl<sub>3</sub>.

> Key words: Aluminum, Ginseng, Radiation, Oxidative stress, Antioxidants.

The impact of Al on human health has been increasingly alarming in recent years. The use of AL utensils may increase one's exposure to AL, particularly when used with salty, acidic or alkaline foods (Sharma and Mishra, 2006). Al and its salts are commonly used in daily life, as it was believed to be non-toxic and quickly excreted in the urine. However, this element, in fact, negatively impacts human health (Osinska *et al.*, 2004). It is present in many manufactured foods (Abbasali *et al.*, 2005) and pharmaceutical products (Roberts *et al.*, 2002).

Al is potentially toxic to humans. It may be a contributing factor for the development of Alzheimer's disease (Campbell, 2002) and skeletal system disease (Gupta *et al.*, 2005).

There is growing evidence in the literature to use some plant extracts that possess an array of interesting pharmacological effects. Ginseng usually refers to the dried root of several species in the plant genus Panax, which belongs to the Araliacceae family (Xie *et al.*, 2004). The putative bioactive components of Panax ginseng are believed to be a mixture of over 30 heterogeneous glycosidal saponins (glycosylated steroids) known as ginsenosides, which are derivatives of the triterpene dammarane structure (Shao *et al.*, 2004). Ginsenosides are associated with a variety of important pharmacological effects in the human body, including antistress (Kennedy *et al.*, 2003) and antidiabetic (Xie *et al.*, 2004). Ginsenosides have also been demonstrated in both humans and rodents to possess bio-modulating and immunomodulating action, and have produced beneficial effects within the cardiovascular, hematopoietic, endocrine, immune and central nervous systems (Kennedy *et al.*, 2003).

The irradiation of ginseng has been considered as a safe, effective and reliable method for preservation and enhancement of the hygienic quality (Cho *et al.*, 1994). Comparative studies of ginseng subjected to  $\gamma$ -rays showed negligible changes in physicochemical attributes (Kwon *et al.*, 2000).

The present study was carried out to investigate the effects of  $AlCl_3$  on some biochemical parameters in rats either in absence or presence of irradiated ginseng in order to demonstrate the potential beneficial role of this medicinal plant against  $AlCl_3$  damage.

## **Materials and Methods**

#### Material

AlCl<sub>3</sub> was purchased from Sigma, chemical Company. Ginseng was purchased from an Egyptian local market (Harraz Co., Cairo, Egypt).

## Irradiation process

Powder of ginseng was transferred into polyethylene bags and treated with 10 KGy of gamma rays, using a  $^{60}$ Co source at a dose rate of 4.75 KGy/ h at the NCRRT, Cairo, Egypt.

White albino male rats  $(200\pm 10g)$  were housed in standard plastic cages at an environmentally controlled room (constant temperature  $25\pm 2^{\circ}C$ , with 12 h light/ dark cycle) during the experiment. They were fed a standard pellet diet and water *ad libitum*.

Four groups of rats each of 8 rats were used; they were treated as follows: Control group: Untreated rats, IGE group: Rats received orally  $\gamma$ -irradiated ginseng powder (400 mg kg<sup>-1</sup> body wt daily) dissolved in water during the experiment which lasted for six weeks (Turan *et al.*, 2010). AL group: Rats received orally by gavages 1 ml of a solution containing 0.5 mg kg<sup>-1</sup> body wt of ALCL<sub>3</sub> in H<sub>2</sub>O per day for six weeks (Al-Hashem, 2009) and IGE + AL group: Rats received IGE + ALCL<sub>3</sub> as described for group 2 and 3.

At the end of the experimental duration (six weeks), the animals were subjected to over night fasting before sacrifice. The rats were euthanized under general anaesthesia with diethyl ether. Blood samples were collected and centrifuged at  $1,500 \times g$  for 10 min to obtain serum.

The counts of red blood cells (RBCs) were counted by the haemocytometer method. The haemoglobin (Hb) level was determined spectrophotometrical and the haematocrit (Hct) volume was determined by the microhaemotocrit tube method. Serum transaminases (aspartate amino transferase; AST) and (alanine aminotransferase; ALT) activities were determined following the method of Reitman and Frankel (1957). Alkaline phosphatase (ALP) activity was measured according to Roy et al (1970). The activity of lactate dehydrogenase (LDH) was measured according to the methods of Moss and Handerson (1994). Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels were estimated using a radioimmunoassay (RIA) kits (Diagnostic Product Corporation, Los Angeles, USA). Also, serum testosterone (T) level was determined using a test reagent kit based on a solid phase enzyme linked immunosorbent assay (Sanchez et al., 1998). Furthermore, thiobarbituric acid reactive substances (TBARS) concentration, (Yoshioka et al., 1979), reduced glutathione (GSH) content (Beutler et al., 1963), superoxide dismutase (SOD) (Minami and Yoshikawa, 1979) and catalase (CAT) (Johansson and Borg, 1988) activities were determined in liver and testes. Moreover, protein content (Lowry et al., 1951).

#### Statistical analysis

Data are given as the mean  $\pm$  SE. One-way analysis of variances ANOVA (Steel and Torrie, 1980) was used to determine if the difference observed among various treatment groups was significant at *P*< 0.05.

## Results

The effect of AlCl<sub>3</sub> and IGE on some haematological parameters of rats and the significance of differences among them are shown on Table 1. After six weeks of AlCl<sub>3</sub> administration, there was a significant decrease in RBCs count, Hb and Hct percentage compared to the control. On the other hand, these parameters were significantly (P< 0.05) increased in IGE+ Al treated group versus AlCl<sub>3</sub> treated rats (Table 1). Significant (P< 0.05) increments in these haematological parameters were also observed when IGE utilized alone.

 TABLE 1. Effect of IGE administration to ALCL<sub>3</sub> intoxicated rats on some haematological parameters.

Groups	<b>RBCs</b> $(10^{6}/\text{ mm}^{3})$	<b>Hb</b> (g/ dl)	Hct (%)
Control	9.28± 0.45 <sup>b</sup>	12.25± 0.35 <sup>b</sup>	43.50± 0.29 <sup>b</sup>
IGE	11.10± 0.02 <sup>a</sup>	$15.05 \pm 0.37^{a}$	$46.70 \pm 0.45^{a}$
Al	$7.40 \pm 0.26^{c}$	$10.56 \pm 0.24^{c}$	$39.00 \pm 1.20^{\circ}$
Al+ IGE	9.18± 0.27 <sup>b</sup>	12.82± 0.10 <sup>b</sup>	42.94± 0.54 <sup>b</sup>

Data are expressed as mean $\pm$  SE. of 8 rats per group. Values with different superscript in the same columns are significantly different at  $P \le 0.05$ .

Significant (P < 0.05) rise was exhibited in the activity of ALT, AST, ALP and LDH in serum of AlCl<sub>3</sub> treated rats relative to control animals. The incorporation of IGE along with AlCl<sub>3</sub> significantly (P < 0.05) reduced the release of these diagnostic hepatic marker enzymes (Table 2).

 

 TABLE 2. Effect of IGE administration on serum liver markers of ALCL3intoxicated rats.

Oroups	ALI $(U/ml)$	AST (U/ ml)	<b>ALP</b> (U/ l)	<b>LDH</b> (U/1)
Control	36.86± 1.73 <sup>c</sup>	$81.27 \pm 2.16^{c}$	77.46± 3.05°	193.44± 4.25 <sup>°</sup>
IGE	36.09± 1.56 <sup>c</sup>	84.08± 1.93 <sup>°</sup>	$76.18 \pm 1.63^{c}$	182.16± 5.78 <sup>d</sup>
Al	49.92± 1.32 <sup>a</sup>	$149.45 \pm 4.86^{a}$	$131.27 \pm 2.72^{a}$	$288.97 \pm 6.13^{a}$
Al+ IGE	$40.69 \pm 1.28^{b}$	97.37± 2.87 <sup>b</sup>	86.34± 1.84 <sup>b</sup>	205.08±4.76 <sup>b</sup>

Legends as in Table 1.

As seen in Table 3, AlCl<sub>3</sub> supplementation to rats produced a significant (P< 0.05) decline in serum T, LH and FSH concentration than control rats. While, the administration of IGE with AlCl<sub>3</sub> for the same treatment period maintained the level of these sex hormones to approximate the control level.

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Groups	T (nmol/l)	<b>LH</b> (U/ l)	<b>FSH</b> (U/1)
Control	$4.73 \pm 0.12^{a}$	$0.88 \pm 0.14^{a}$	$0.79 \pm 0.05^{a}$
IGE	$4.87 \pm 0.15^{a}$	$0.86 \pm 0.03^{a}$	$0.76 \pm 0.06^{a}$
Al	2.52± 0.11 <sup>b</sup>	$0.51 \pm 0.02^{c}$	$0.61 \pm 0.06^{b}$
Al+ IGE	$4.67 \pm 0.13^{a}$	$0.75 \pm 0.02^{b}$	$0.71 \pm 0.04^{ab}$

TABLE 3. Effect of IGE administration on serum level of T, LH and FSH in AlCl<sub>3</sub> intoxicated rats.

Legends as in Table 1

As a consequence of exposure of rodents to  $AlCl_3$ , it could be noticed that there was a significant (P < 0.05) elevation in the concentration of hepatic and testicular TBARS accompanied with a depression in GSH content in comparison with control rats. Concomitant oral administration of IGE and  $AlCl_3$ restored the TBARS and GSH content near the control levels (Table 4).

TABLE 4. Effect of IGE administration on the tissues TBARS and GSH levels in rats exposed to ALCL<sub>3</sub>.

	<b>TBARS</b> (n mol/ g tissue)		<b>GSH</b> (mg/ g tissue)	
Group	Liver	Testes	Liver	Testes
Control	$159.28 \pm 2.62^{\circ}$	$137.54 \pm 7.03^{\circ}$	$57.38 \pm 1.44^{a}$	$27.11 \pm 2.29^{a}$
IGE	$153.31 \pm 4.92^{\circ}$	$102.49 \pm 3.97^{d}$	$58.40 \pm 2.59^{a}$	$27.24 \pm 1.5^{a}$
Al	$221.25 \pm 5.98^{a}$	$198.26 \pm 9.24^{\rm a}$	$26.52 \pm 2.87^{\circ}$	$18.92 \pm 1.13^{\circ}$
Al + IGE	$176.08 \pm 5.84^{b}$	$161.10 \pm 4.65^{b}$	$50.25 \pm 2.1^{b}$	$23.84 \pm 1.12^{b}$

Legends as in Table 1.

SOD and CAT activity in the rat's liver and testes were significantly (P < 0.05) diminished in the Al group concerning control rats. However, feeding rats IGE alleviated the effects of AlCl<sub>3</sub> and resulted in a significant (P < 0.05) increase in the antioxidant activity of these two enzymes when compared to Al.

 TABLE 5. Effect of IGE administration to ALCL<sub>3</sub> intoxicated rats on SOD and CAT activity of liver and testes.

Group	<b>SOD</b> (U/ mg protein)		<b>CAT</b> (U/ mg protein)	
	Liver	Testes	Liver	Testes
Control	51.19±3.37 <sup>a</sup>	19.66±1.44 <sup>a</sup>	$15.81 \pm 0.27^{a}$	$11.88 \pm 1.2^{a}$
IGE	52.12±1.64 <sup>a</sup>	$20.79 \pm 0.86^{a}$	$16.07 \pm 0.11^{a}$	12.14±0.83 <sup>a</sup>
Al	$26.04 \pm 1.62^{b}$	13.83±0.76 <sup>c</sup>	10.38±0.13 <sup>c</sup>	$8.97 \pm 0.86^{\circ}$
Al + IGE	$48.88 \pm 3.92^{a}$	$17.65 \pm 1.27^{b}$	13.74±0.34 <sup>b</sup>	$10.61 \pm 0.54^{b}$

Legends as in Table 1.

## Discussion

The present study was undertaken to determine whether IGE can prevent or reduce Al-induced oxidative stress in rats. AL toxicity may be mediated by

free radical generation and alterations in antioxidant enzymes in vivo and in vitro (Tabaldi *et al.*, 2009). The significant (P < 0.05) decreases in Hb, RBCs and Hct among AL-treated rats corroborate the findings of Abdel Aziz and Zabut (2011). The reduction in Hb content might be due to increased rate of destruction or reduction in the rate of formation of RBCs. This intepretation was supported by the low levels of RBCs in the treated group. Vittori *et al.* (2002) have reported that AL may disturb erythropoiesis through combined effects on mature erythrocytes and cellular metabolism in late erythroid progenitors. Reductions in Hct, RBCs and Hb might be attributed to hyperactivity of bone marrow, leading to production of RBCs with impaired integrity that easily destroyed in the circulation (Karmakar *et al.*, 2000). The decline in Hb could be not only due to decrease in RBCs count but also to impaired biosynthesis of haeme in the bone marrow (Karmakar *et al.*, 2000).

As seen here, co administration of IGE with AL showed beneficial haematological effects. The RBCs, Hb and Hct values were largely restored. In harmony with these observations, it was deduced that ginseng improved the haematological parameters of rats (Karadeniz and Altintas, 2008). This protective effect of ginseng against AL-induced toxicity can be ascribed mainly to its antioxidant ability or its stimulatory effects on erythropoiesis (Scaglione *et al.*, 1990). Moreover, panax ginseng treatment may stimulate the activity of the bone marrow stem cells and consequently strengthen systemic and particularly immune cellular defences of the organism (Jensen *et al.*, 2000).

The activities of AST, ALT, ALP and LDH in serum were increased after toxicant administration. These findings are well correlated with those of Abdel Aziz and Zabut (2011) and Al-Hashem (2009). It is of interest to mention that in animals treated with toxicants, the serum levels of these enzymes elevated after liver damage, and thus alterations in liver function, because of increased membrane permeability or due to liver cell necrosis and cytosol leakage into the serum (Ozer *et al.*, 2008). The data of the current study revealed that the incorporation of IGE inhibited the rise in serum level of these enzymes. This could indicate improvement of liver function and protection from the toxicity of AL. These results are in agreement with the study of Young *et al.* (2011) who mentioned that Korean red ginseng (KRG) pre-treatment clearly ameliorated the increased levels of ALT and AST indicating that KRG may have protective effects against hepatotoxicity induced by Aflatoxin B1. As well, *Egypt. J. Rad. Sci. Applic.*, Vol. 24, No. 2 (2011)

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Hye *et al*, (2011) postulated that KRG treatment prior to administration of ethanol (EtOH) to rats decreased the EtOH-induced increase in serum AST, ALT and LDH.

The obtained findings proved that AlCl<sub>3</sub> intoxication to rats significantly (P < 0.05) reduced serum T, FSH and LH levels. These data go in line with those of Shahraki and Palan (2006). Those authors documented that AL injection in rats has an adverse effect on some sex hormones. As well as, Wang et al. (2012) sustained that  $AlCl_3$  administrated orally to rats significantly diminished the levels of T, FSH, and LH. In this regard, IGE along with AlCl<sub>3</sub> produced a significant (P < 0.05) enhancement in T and FSH levels. The LH level was also improved. These outcomes are consistent with the earlier report of (Fahim et al., 1982). Panax ginseng is an old and well-known plant that is used to prevent sexual dysfunction. It was suggested that the antioxidant and organ protective actions of ginseng are associated with enhanced nitric oxide (NO) synthesis in the endothelium of the lung, heart, kidney and corpus cavernosum (Chen and Lee, 1995). Enhanced NO synthesis causes vasodilatation and might be responsible for the aphrodisiac property of ginseng (Murphy and Lee, 2002). Treatment with ginseng has been shown to significantly alter the activity of hypothalamic catecholamines involved in the facilitation of hormone secretion (Murphy and Lee, 2002). They also indicated that ginseng improves the reduced feedback from the testes to the pituitary gland, resulting in an increase in the amount of testosterone secreted from LHstimulated Leydig cells.

Chronic exposure to AlCl<sub>3</sub> causes a mineral imbalance in which AL ions replace iron and magnesium ions resulting in a reduction of Fe2+ binding to ferritin (Ward *et al.*, 2001). Free iron ions released from biological complexes by AL can catalyze hydrogen peroxide, generating hydroxyl radicals through Fenton's reaction (Ward *et al.*, 2001). These radicals are able to initiate LPO and cellular damage (Yousef *et al.*, 2007). Also, AL has been reported to promote non-iron-induced LPO (Ward *et al.*, 2001). Experimental data exhibited that toxicant supplementation to rats significantly (P< 0.05) increased the LPO in liver and testes which was evident by the increased production of TBARS. In agreement with these issues, Shrivastava (2011) and Al-Hashem (2009) declared significant increase in TBARS in the kidney, liver, brain and

testes of rats after intoxication by AL salts. The enhanced TBARS suggests participation of free-radical induced oxidative cell injury in mediating the toxicity of Al. High LPO is, at least in part, due to an inhibition or alteration in the activity of non-enzymatic and enzymatic components of the oxidative system.

GSH, an essential component of oxidative system, serves as a cofactor for glutathione transferase, which helps to remove certain drugs and chemicals, as well as reactive molecules, from the cells (Wu and Cederbaum, 2003). Moreover, GSH can directly detoxify hydroxyl radicals and is critical for mediating other key activities in the cell. Al caused significant (P < 0.05) decrease in the GSH content in liver and testes. Metals as cadmium, cisplatin, lead and mercury induced oxidative stress by depleting the major intracellular antioxidant, glutathione in liver, kidney and brain (Afifi, 2010). Al might affect GSH synthesis by decreasing the activity of glutathione-synthase thus leading to a reduced GSH levels (Orihuela *et al.*, 2005).

IGE plus AlCl<sub>3</sub> produced significant (P < 0.05) decrease in TBARS accompanied by significant (P < 0.05) increase in GSH content. In accordance with these observations Zhang *et al.* (2008) concluded that ginseng total saponin was shown to protect against oxidative stress induced by cyclophosphamide in mouse bone marrow cells and peripheral lymphocytes. Also, Gum *et al.* (2007) reported that ginseng water extract reversed the reduction of GSH and GST activity induced by benzo [a] pyrene in rats.

The enzymatic antioxidant defence system, which includes SODs and CATs, helps protect cells from oxidative injuries. SOD catalyzes the rapid removal of superoxide radicals, generating  $H_2O_2$ , which is eliminated by catalases (Wu and Cederbaum, 2003). In the present work, AlCl<sub>3</sub> induced free radicals and may inhibit the enzymes involved in antioxidant defense: SOD and CAT. A significant drop was observed in the serum antioxidant enzymes of treated rats. This coincides with Nehru and Anand (2005) who stated a significant reduction in the activities of SOD and CAT in the brain after AL treatment. The decline in both enzyme activities could be due to a reduced synthesis of these enzymes proteins because of higher intracellular concentrations of AL or due to accumulation of free radicals (Nehru and Anand, 2005).

Significant (P < 0.05) rise in the two antioxidant enzymes in the liver and testes of treated rats were observed in response to combined treatment of IGE and AlCl<sub>3</sub>. Kim *et al.* (2005) ascertain that ginseng extract produced elevation of CAT and SOD activity and decreased malondialdehyde levels in sedentary male patients. Recently, Young *et al.* (2011) corroborated these events. Ginseng root extracts exhibit both lipid-soluble and water-soluble antioxidant activity ex vivo, and that this antioxidant action occurs both directly through free radical scavenging and indirectly through up regulation of antioxidant enzymes (Kim *et al.*, 2002). Ginsenosides have been associated with the up regulation of both SOD and CAT enzymes at the level of gene expression and transcription (Kim and Park, 2003).

Our results demonstrate that  $AlCL_3$  alters haematological, biochemical and hormonal parameters and induce oxidative stress. Consequently, attention should be paid to the sources of AL in food, water and medical drugs. Ginseng ingestion along with  $AlCL_3$  exposure minimized Al-associated hazards due to its antioxidant and free radical scavenging properties. Therefore, ginseng could be beneficial for reducing AL toxicity.

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الدور المحتمل للجينسينج المعامل بأشعة جاما فى تعديل بعض الاضطرابات الكيميائية الحيوية الناتجة عن الألمونيوم فى الجرذان

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قسم بحوث تشعيع الأغذية ، المركز القومي لبحوث وتكنولوجيا الإشعاع ، ص . ب ٢٩ مدينة نصر ، القاهرة ، مصر.

لقد حظي التأثير السام للألمونيوم في الإنسان والحيوان على قدر كبير من الاهتمام. يتعرض الجينسينج للتلوث بالميكر وبات أثناء التداول قبل وبعد الحصاد. استخدمت أشعة جاما لإزالة هذا التلوث وذلك لضمان السلامة الميكروبيولوجية. تهدف هذه الدراسة الى معرفة ما إذا كـان للجينسينج المعامل اشعاعيا (١٠كيلوجراى) تأثير واقي من السمية التي قد يحدثها الألمونيوم في الجرِّذان. قسمت ذكور الجرذان آلي أربعة مجمو عات تحتوى كل منها على ثمانية جر ذان: مجموعة ضابطة، مجموعة الجينسينج تناولت المستخلص المائي للجينسينج المعامل بأشعة جاما بجرعة يومية ٤٠٠ مليجرام/ كجم وزن جسم عن طريق الفم ، مجموعة الألمونيوم تناولت كلوريد الألمونيوم بجرعة يومية (٥٠ ماليجرام/ كجم وزن جسم) عن طريق الفم ، مجموعة الجينسينج و الألمونيوم تناولت كل من المستخلص المائي للجينسينج المعامل بأشعة جاما و كلوريد الألمونيوم. استمرت الفترة التجريبية ست أسابيع. تسبب الألمونيوم في حدوث نقص معنوي في بعض معابير الدم وكذلك الهرمونات الجنسية وتثبيط فعاليات بعض الانزيمات المضادة للأكسدة في الكبد والخصية ، صاحب ذلك زيادة معنوية في الأنزيمات الكبدية والأكسدة الدهنية بالأنسجة. عندما تناولت الجرذان المستخلص المائي للجينسينج المعامل بأشعة جاما و كلوريد الألمونيوم معا عادت كل هذه القياسات السابقة تقريبا لمستوى المجموعة الضابطة. أكدت هذه النتائج أن تناول مستخلص الجينسينج المعامل بأشعة جاما فعال في الحماية منَّ سمية الألمونيوم.