

Online ISSN: 2682-2628
Print ISSN: 2682-261X

IJC CBR

INTERNATIONAL JOURNAL OF CANCER AND BIOMEDICAL RESEARCH

<https://jcbr.journals.ekb.eg>

Editor-in-chief

Prof. Mohamed Labib Salem, PhD

**Effect of *Moringa oleifera* on antioxidant enzymes
and oxidative stress induced by aluminium
exposure in male albino rat testes**

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PUBLISHED BY

EACR EGYPTIAN ASSOCIATION
FOR CANCER RESEARCH

Since 2014

Effect of *Moringa oleifera* on antioxidant enzymes and oxidative stress induced by aluminium exposure in male albino rat testes

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ABSTRACT

This study summarizes the effect of aqueous leaves extract of *Moringa oleifera* on oxidative stress-induced from exposure to aluminium (aluminium chloride, $AlCl_3$) on testes of male albino rats. Fifteen male albino rats (250.0 ± 10.0 gm) were divided into five groups, each group of 10 rats. By means of gastric tube group one received sterile water as a control, group two received $AlCl_3$ (50mg/kg/day), group three received *M. oleifera* leaves extract (300mg/kg/day), group four received $AlCl_3$ (50mg/kg/day) for four weeks then *M. oleifera* (300mg/kg/day) for four weeks, and group five received $AlCl_3$ and *M. oleifera* for eight weeks. The results showed significant changes in testes parameters where there was a decrease in the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione -s- transferase (GST) and increase in the activity of xanthine oxidase (XO) and malondialdehyde (MDA) levels in aluminium treated groups. There was an improvement in the activity of the enzymes (SOD, CAT, GPx, and GST) associated with a decrease in the activity of XO and MDA in groups received *M. oleifera*. The results proved that *M. oleifera* has an ameliorative effect on the activities of the antioxidant enzymes and the oxidative stress-induced from exposure to aluminium in the testes of male albino rats.

Keywords: Aluminium toxicity, *Moringa oleifera* leaf extracts, Antioxidant enzymes, Oxidative stress, Rat testes

Editor-in-Chief: Prof. M.L. Salem, PhD - Article DOI: 10.21608/jcbr.2019.65450

ARTICLE INFO

Article history

Received: November 15, 2019

Revised: December 10, 2019

Accepted: December 17, 2019

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INTRODUCTION

Aluminium is one of the ubiquitous metals that is being immensely used in industries, pharmaceuticals, food additives, and consumer products. Concomitantly, there has been an increased incidence of exposure to the general population which can cause serious effects on various systems of the body including male reproductive system (Geeta & Gyan .2017).

Moreover, high levels of aluminium in spermatozoa and seminal plasma of humans have been reported to reduce sperm viability and motility (Guo *et al.*, 2005 and Yousef *et al.*, 2007). Also, aluminium may cause male reproductive toxicity through various mechanisms such as inducing oxidative stress, interfering with spermatogenesis and disrupting the blood-testes barrier (Geeta & Gyan, 2013). Reproductive toxicity is a major challenge associated with aluminium exposure. Aluminium causes reproductive dysfunction by

creating oxidative damage. It damages the testicular tissues of humans and animals.

Testicular aluminium accumulation necrosis of spermatocytes/spermatids and a significant reduction in fertility were also observed in both male rats and mice (Sharma *et al.*, 2003 and Guo *et al.*, 2005). The increment of intake of antioxidants would ameliorate the damage caused by oxidative stress through inhibiting the initiation or propagation of oxidative chain reaction, acting as free radical scavengers (Dong-Ping *et al.*, 2017). Antioxidant defences such as antioxidant enzymes and antioxidant food ingredients play an important role in scavenging free radicals and delaying the progress of many long-lasting diseases in aerobic organisms. These compounds may be created in the body or taken from food (Santos-Sanchez *et al.*, 2017).

Phytochemical antioxidants are used to maintain health and avoid diseases (Bhalla *et al.*, 2010, Azzini *et al.*, 2017 and Mangge *et al.*, 2017). Natural

antioxidants could be used to decline free radicals created from harmful effects (Seifi *et al.*, 2018). Some studies proved the benefits of *M. oleifera* as an antioxidant (Anwar *et al.*, 2007). This tree is the greatest broadly scattered species of the Moringaceae family all over the biosphere having an amazing variety of pharmacological properties in addition to its important dietary value (Ekong *et al.*, 2017). Also, it was described to be used against metal intoxication (Khatum & Varma, 2018). The numerous plant parts have wide medicinal applicability for the handling of cardiovascular diseases as the roots, leaves, gum, and flowers (Jaja-Chimedza *et al.*, 2018 & Rouhi-Boroujeni *et al.*, 2017). Seed infusion contains nitrite, mustard oil glycosides and thiocarbamate glycosides as their significant bioactive ingredients that are thought to be responsible for urinary excretion, cholesterol-lowering and antiulcer properties (Cuellar-Nunez *et al.*, 2018).

The leaves extract of *M. oleifera* and other parts have been shown to have a powerful antioxidant action *in vivo* (Jinghua *et al.*, 2018 & Fakurazi *et al.*, 2012). Based on our information, the studies on the effect of ethanolic extract of leaves of *M. oleifera* to ameliorate the oxidative stress action in the testes of white albino rats induced by $AlCl_3$ intoxication are not enough. So, this study was directed to evaluate the effect of aluminium on the activity of SOD, CAT, GST, GPx, XO, and MAD in the testes of male albino rats and co-supplementation of the aqueous leaves extract of *M. oleifera* (AMLE) herbal antioxidant *M. oleifera*.

MATERIALS AND METHODS

The experimental animals

Fifty young male albino rats Sprague- Dawley (S.D.) weighting 250.0 ± 10 g. purchased from the holding company for biological product and vaccines (VACCERA), Helwan, Egypt, were used as experimental animals. The animals were housed in plastic mesh cages and acclimated for one week in the laboratory. They were maintained at $25 \pm 2^\circ C$ with relative humidity $55 \pm 5\%$ at the normal light-dark cycle. All animals were given ad libitum access to natural food and water. The experiment was done according to the national regulations in animal welfare and institutional Animal Ethical Committee (IAEC). Animals were

carefully put under observation every day to pursue any signs of abnormalities and toxicity during the experiment.

The experiment

Rats were divided into five groups, each one of 10 rats. The animals were given doses by stomach tube and the experiment remained eight weeks. Aqueous *M. oleifera* leaves extract (AMLE) was prepared at room temperature according Attah *et al.* (2019). Group 1 (control) animals were daily received tap water for eight weeks. Group2 ($AlCl_3$) animals were daily received $AlCl_3$ for eight weeks with 50.0 mg/kg/day dose (Khatum & Varma, 2018). Group 3 animals were daily received AMLE for eight weeks with the dose 300mg/kg/day. Group4 animals were daily received $AlCl_3$ for four weeks with the dose 50.0mg/kg day then daily received AMLE for four weeks daily with the dose 300mg/kg/day. Group 5 animals were daily received for eight weeks $AlCl_3$ with the dose 50.0 mg/kg/day, and AMLE with the dose 300mg/kg/day.

Methods

Tissue preparation

At the end of the experiment after 8 weeks, all animals were fasted overnight and sacrificed after diethyl ether anesthesia. Testes were immediately excised, washed with ice- cold physiological saline (0.9 gm % NaCl) to remove blood and blotted to dry, weighted. Tissue samples were homogenized in ice-cold phosphate buffer (50 mM phosphate pH 7.4) 10% (w/v) using Ommi international homogenizer (USA) at 22.000 rpm for 20s. each with 10s. intervals. The homogenate was centrifuged at 2000 Xg in the cooling centrifuge (Hettich, Germany) at $4^\circ C$ for 15min. and the supernatant was saved. The supernatant was freeze-thawed twice to complete mitochondrial disruption (Salach Jr, 1978). The supernatant was again centrifuged at 6000Xg at $4^\circ C$ for 15min. and the yielded supernatant which contains the cytosolic and mitochondrial enzymes was saved for immediate enzyme assays.

Enzymes were assayed by using the Automated Elisa System (Chemwell 2099 from Gama Trade company). The research kits for application type of ELISA, Kamiya Biomedical Company (Catalog no. KT-50849) was used for SOD assay activity. The

research enzyme kits for application type of ELISA, my BioSource (Catalog no. 038818,96 th) was used for CAT activity assay. The research enzyme kits Bioassay Laboratory Technology for application type of ELISA (Catalog no. E1172RA) was used for GPx activity assay, (catalog no. E0943Hu) was used for GST activity assay, (Catalog no. E3495ttu) was used for XO activity assay and (Catalog no. KT-53246) was used for MDA activity measurement.

Statistical analysis

Data were analyzed and represents mean \pm standard deviation ($X \pm SD$) of 10 rats. Statistical comparisons were performed using one-way analysis of variance (ANOVA) followed activity of male albino rat testes by Dunnett's test to compare mean values between treatment groups and control. The $P \leq 0.05$ or less value was considered statistically significant using a computer program (GraphPad InState Software. Inc.).

RESULTS

The ameliorative role of *M. oleifera* on the oxidative stress-induced from aluminium exposure was obvious, and this is noticed in figures (1-5). Data were presented as five columns, control group, aluminium treated group, *M. oleifera* treated group, administration of *M. oleifera* for four weeks after administration of aluminium for four weeks and the last group present administration of *M. oleifera* along with aluminium for eight weeks. The activity of SOD, CAT, and GPx in testes showed a significant decrease in group 2 and 4 compared with the control group. The activity of GST in testes showed a significant decrease in group 2 compared with the control group. The activity of XO in testes showed a significant increase in group 2 and 4 compared with the control group. The level of MDA concentration in testes showed a significant increase in group 2 and 4 compared with the control group.

DISCUSSION

In the present study, there was a decrease in the activity of SOD, CAT, GPx, and GST parallel with an increase in the activity of XO and level of MAD concentration. This agrees with (Guo *et al.*, 2005), who proved the ability of aluminium to cause oxidative stress, cross the blood-testes barrier,

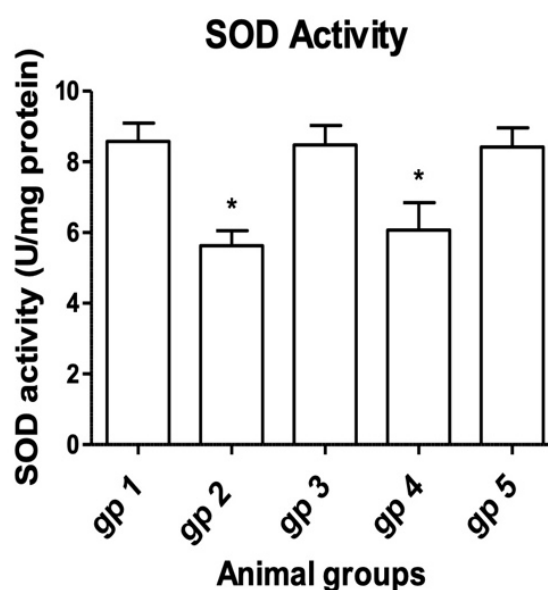


Figure 1. Effect of AMLE on aluminium on SOD

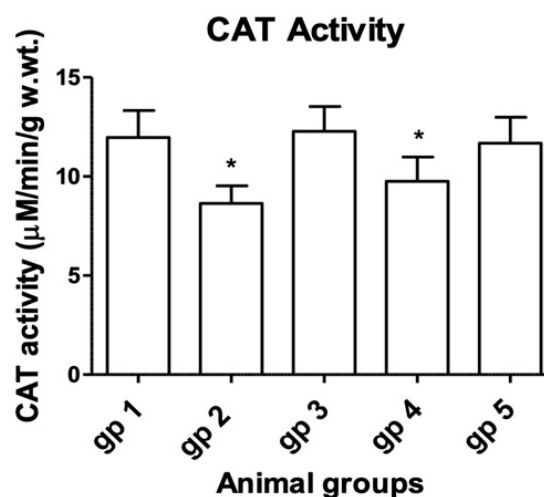


Figure 2. Effect of AMLE on aluminium on CAT activity of male albino rat testes

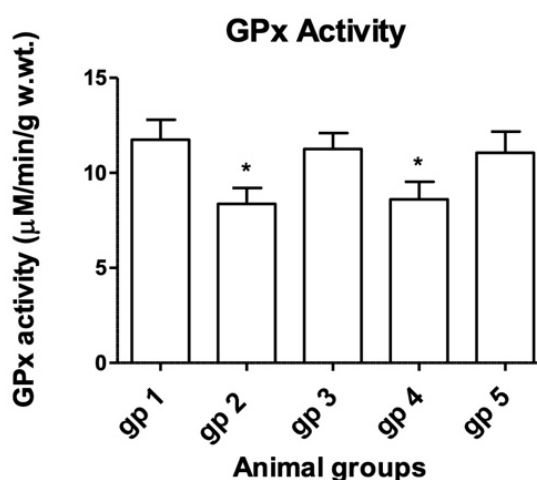


Figure 3. Effect of AMLE on aluminium on GPx activity of male albino rat testes

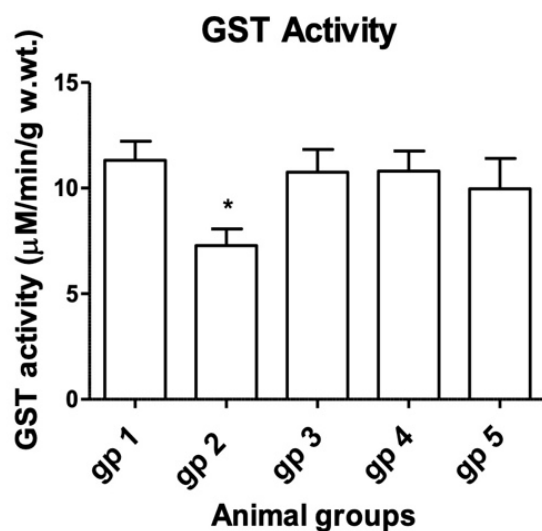


Figure 4. Effect of AMLE on aluminium on GST activity of male albino rat testes

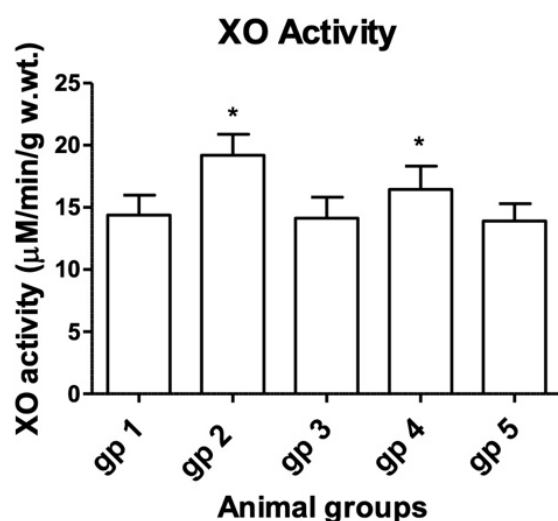


Figure 5. Effect of AMLE on aluminium on XO activity of male albino rat testes

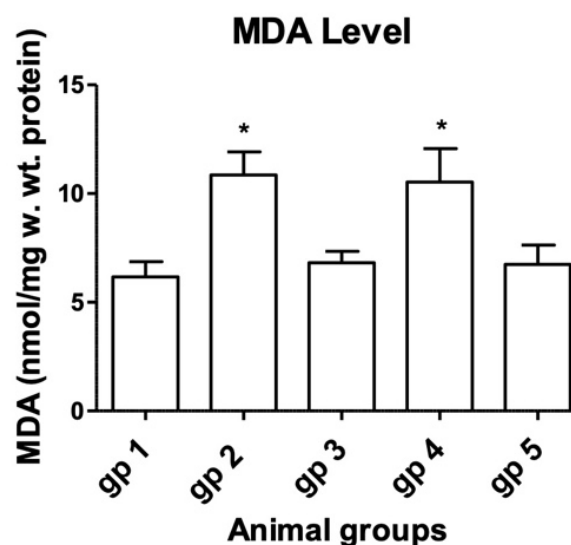


Figure 6. Effect of AMLE on aluminium on MDA level of male albino rat testes

promote lipid peroxidation. This is similar to the results reported by Ebrahim *et al.* (2016) who proved the oxidative stress-induced from aluminium in testes of Wistar rats and cause alteration in the antioxidant enzymes.

Turgut *et al.* (2006) reported that aluminium exposure can increase MDA level as it interacts with cell membrane directly as aluminium salt. This may accelerate lipid peroxidation in membrane lipids induced by Fe (II) salts. Aluminium ions produce a subtle rearrangement in the membrane structure that causes the oxidative action of iron (Hosny *et al.*, 2018). Savory *et al.*, 2003 found that aluminium cytotoxicity may be mediated by free radicals derived from this element and its capability to induce oxidative stress through a wide variety of mechanisms including production of reactive oxygen species (ROS). This agrees with the results of this study which proved the decrease of the activity of SOD, CAT, GPx, and GST in aluminium treated rat testes.

In the present study, the co-supplementation of the herbal antioxidant aqueous *M. oleifera* extract (AMLE) reduces the damaging effects of aluminium-intoxication, on the activity of these enzymes and lipid peroxidation. The AMLE caused improvement in the activities of SOD, CAT, GPx, and GST in testes and these results resembles the findings of Al-Amoudi, (2018) who found an ameliorative effect of ginger extract on toxic effects of Lambda-cyhalothrin on in the thyroid of rat.

The enzyme XO is considered an important biological source of superoxide radical (Hegazi *et al.*, 2015). When acting as NADH oxidase, XO is a generator of superoxide, a powerful ROS. These reactive oxygen species affect various molecular components of the cell, with excess amounts leading to cell degeneration and death. Studies on XO have shown that modulation of enzyme activity, cofactor availability, substrate concentration, and oxygen tension all affect rates of intracellular ROS production (Stanton *et al.*, 2017). In this study, an increase in the activity of XO may be due to an increase of ROS generation as a result of exposure to aluminium. The AMLE ameliorated the activity of the antioxidant enzymes due to its ability to scavenge ROS by the extract constituents (Patel *et al.*, and

Jaja-Chimedza *et al.*, 2018). At last, this study indicated that aluminium- intoxication can affect testes causing oxidative stress and changes in the rearrangement of lipids causing lipid peroxidation which leads finally to cell death, and AMLE can limit this oxidative stress. Aluminium might have been responsible for reductions in sperm motility and viability. It causes morphological abnormalities (Pandey & Gyasn, 2013).

CONCLUSION

This study indicated that aluminium-intoxication can affect testes causing oxidative stress and changes in the rearrangement of lipids causing lipid peroxidation which leads finally to cell death, and AMLE can limit this oxidative stress.

CONFLICT OF INTEREST

There is no conflict of interest.

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