Ameliorative effect of *Moringa oleifera* on oxidative stress in male albino rat brain promoted by aluminium exposure

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Abstract: This study evaluates the effect of *Moringa oleifera* leaf extract on oxidative stress after chronic exposure to aluminium (aluminium chloride, $AlCl_3$) on brain in male albino rats. Male rats (250.0±10.0 g) were separated into five groups of ten animals each. Group 1 served as control, group 2 supplemented with $AlCl_3$ dose 50 mg/Kg/day, group 3 supplemented with *M. oleifera* at dose of (300 mg/Kg/day), group 4 supplemented with $AlCl_3$ for 4 weeks then *M. oleifera* for 4 weeks and group 5 supplemented with $AlCl_3$ and *M. oleifera* for 8 weeks. The results showed significant changes in brain parameters. Supplementation of *M. oleifera* as antioxidant improves the activity of the enzymes superoxide dismutase (SOD), catalase (CAT), Glutathione-S-transferase (GST), glutathione peroxidase (GPx), monoamine oxidase (MAO) and acetylcholinesterase (AChE), and decrease in the activity of the enzymes xanthine oxidase (XO), and creatine kinase (CK) and malondialdehyde (MDA) level in aluminium treated groups. The results point to that *M. oleifera* is a potent antioxidant has ameliorative effect against changes in antioxidant enzymes and oxidative stress biomarker in rat brain.
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1. Introduction

Aluminium-intoxication generates oxidative stress through lipid and protein oxidation and oxidative stress has been associated with Alzheimer disease (AD) (Azzini *et al.*, 2017). Alzheimer disease represents a neurodegenerative disease leading to progressive dementia in the elderly. The presence of aluminium in dialysis fluids has been shown to be an etiological factor contributing to several neurological disorders known as dialysis dementia (Nayak *et al.*, 2016). Hence, markers for oxidative stress are expected to accumulate not only in brain mitochondria, but also in mitochondria of peripheral cells (Bhalla *et al.*, 2010). Endogenous antioxidant defenses of enzymatic and non-enzymatic nature are vital for the control of the oxidative destruction of molecules e.g. DNA, RNA, protein and membrane lipids (An *et al.*, 2015, Temiz *et al.*, 2015, Carvalho-Silva *et al.*, 2017).

All aerobic organisms have antioxidant defenses, containing antioxidant enzymes and antioxidant food ingredients, to get rid of the injured molecules. Antioxidant compounds can scavenge free radicals and raise shelf life. They delay the progress of many long-lasting diseases. These compounds may be created in the body or got from the food (Santos-Sánchez *et al.*, 2017). Therefore, there is a big need to find other natural and safe sources of food antioxidant. Due to the importance of herbal antioxidants, the world is looking for a variety of natural sources and alternative to the antioxidants created (Hassan *et al.*, 2015, Sushma *et al.*, 2006, Corte and Stirpe, 1972, Moumen *et al.*, 2001, Baranisrinivasan *et al.*, 2009, Ravera *et al.*, 2010, Liu *et al.*, 2010). Phytochemical antioxidants are used as components in nutritional additions to maintain health and avoid diseases (Mangge *et al.*, 2017, Azzini *et al.*, 2017, Linardaki *et al.*, 2013, Bhalla *et al.*, 2010, De la Torre *et al.*, 2000). Natural antioxidants could be used to decline free-radical-created from harmful effects (D'angiolillo *et al.*, 2018, Seifi *et al.*, 2018). Antioxidant phytochemicals, e.g. vitamins C, E, glutathione, flavonids and vegtable dyes, can offer protection against cellular damage (Giri *et al.*, 2017).

The herb *Moringa oleifera*, which has shown some recent studies the benefits of it as antioxidant (Anwar *et al.*, 2007, Falowo *et al.*, 2017). Antioxidants are categorized into two broad partitions, according to whether they are soluble in water (hydrophilic) or in lipids (hydrophobic). In general, water-soluble antioxidants react with oxidants in the cell cytosol and the blood plasma, while lipid-soluble antioxidants protect cell membranes from lipid peroxidation (Jongberg *et al.*, 2017). *Moringa oleifera* 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 important dietary value (Ekong *et al.*, 2017). *M. oleifera* is a highly appreciated herbal in tropic and subtropical nations wherever it is generally cultivated. *M. oleifera* was described to be used against metal intoxication e.g. arsenic lead (Khatun

and Varma, 2018, Mallya *et al.*, 2017, Brraich and Kaur, 2017). It similarly reported to show progressive anti-lead ameliorative properties with neuroprotective effects in focal cerebral ischemia (Kamble *et al.*, 2017, Ekong *et al.*, 2017, Igado *et al.*, 2018). The numerous plant parts have wide medicinal applicability for the handling of cardiovascular diseases as the roots, leaves, gum, flowers (Jaja-Chimedza *et al.*, 2018, Rouhi-Boroujeni *et al.*, 2017). Seed infusion contain nitrile, mustard oil glycosides, and thio-carbamate glycosides as their significant bioactive ingredients, which are thought to be responsible for urinary excretion, cholesterol lowering, and antiulcer properties (Dutta, 2017, Mishra *et al.*, 2011, Cuellar-Nuñez *et al.*, 2018). *M. oleifera* leaf extract when taken alone has not any histologically noticeable disorderly or damaging effects on the cerebral cortex (Ekong *et al.*, 2017).

The strong *in vitro* antioxidant properties of the methanolic extract of *M. oleifera* eatable parts. Leaves of *M. oleifera* have highly essential nutrition such as protein, β-carotene, vitamins A, B, C and E, riboflavin, nicotinic acid, folic acid, pyridoxine, amino acids, minerals and various phenolic compounds (Maizuwo *et al.*, 2017, Sharma *et al.*, 2018). This antioxidant activity of *M. oleifera* extracts is due to the presence of various bioactive compounds such as chlorogenic acid, rutin, quercetinglucoside, and kaempferolrhamnoglucoside (Nascimento *et al.*, 2016). Also, the extract of *M. oleifera* leaves and other parts have been shown to have powerful antioxidant action *in vivo* (Murray, 2003, Singh *et al.*, 2014, Jinghua *et al.*, 2018, Fakurazi *et al.*, 2012). To our knowledge, there are no enough studies on the oxidative stress status induced by toxic substances as $AlCl_3$on rats. For that reason, this study was directed to examine the effect of ethanolic extract of leaf of *M. oleifera* on the oxidative stress action on brain in white albino rat induced by AlCl3 intoxication.

2. Materials and methods

The experimental animals

Fifty young male albino rats Sprague-Dawley weighting 251.0 ± 10 g. The rats were obtained from the Holding Company for Biological Products & Vaccines (VACCERA), Helwan, Egypt. The rats were housed in plastic mesh cages for one week before the beginning of the experimental work that hang about for 8 weeks. The institutional animal care and use facilities from the Zoology Department, Faculty of Science, Tanta University-Egypt, approved the experimental design. Animals were fed on tap water supplied *ad libitum*. The temperature in the animal room was upheld at 25±3℃with a relative humidity of 55±5% at the normal light-dark cycle. The experiment was done according to the National regulations on Animal Welfare and Institutional Animal Ethical Committee (IAEC).

Animals were carefully observed every day. Their body weights, food consumptions, and water intake were registered precisely every week to follow up any signs of toxicity or abnormality during the experiment.

The experiment

Rats were divided into five groups, each group of 10 rats. The Animals were given doses by stomach tube.

- Group 1: (control) animals were daily received tap water for eight weeks.
- Group 2: ($AlCl_3$) animals were daily received ($AlCl_3$) for eight weeks with 50.0 mg/kg/day dose (Yassa *et al.*, 2017, Sun *et al.*, 2017).
- Group 3: (*M. oleifera* extract) animals were daily received (*M. oleifera*) for eight weeks with 300 mg/kg/day dose (Khatun and Varma, 2018).
- Group 4: (therapeutic one) animals were daily received ($AlCl_3$) for four weeks with 50.0 mg/kg/day dose then *M. oleifera* for four weeks daily with 300 mg/kg/day dose.
- Group 5: (therapeutic two) animals were daily received for eight weeks ($AlCl_3$) with 50.0 mg/kg/day dose and *M. oleifera* with 300 mg/kg/day dose.

Methods

Tissue preparation

Liver and brain, were immediately taken out, washed with ice cold saline to remove blood and blotted to dry, weighed and kept at – 80.0 ℃.

Tissue samples were homogenized in ice-cold phosphate buffer (50 mM phosphate pH 7.4) 10% (w/v) using Omni international homogenizer (USA) at 22,000 rpm for 20 s each with 10 s intervals. The homogenate was centrifuged at 2000 Xg in cooling centrifuge (Hettich, Germany) at 4ºC for 15 min and the supernatant was saved. The supernatant was freeze-thawed twice to complete mitochondria disruption (Salach Jr, 1978). Then the supernatant was again centrifuged at 6000Xgat 4℃ for 15 min and the yielded supernatant which contains the cystolic and mitochondrial enzymes was saved for immediate enzymes assays.

Enzymes and MDA were assayed by using 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, and (catalog no. KT-53246) was used for MDA level determination. The research enzyme kits for application type of ELISA, myBioSource (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. E3495Hu) was used for XO activity assay (catalog no. E774Hu) was used for MAO activity assay (catalog no. E0724Ra) was used for AChE activity assay and (cat. no. E3813Hu) was used for CK activity assay.

Statistical analysis

Data were analyzed and represents mean±standard deviation (X±SD). Statistical comparisons were performed using one-way analysis of variance (ANOVA) followed by Dunntt's test to compare mean values between treatment groups and control. A value of $P \leq 0.05$ was considered as statistically significant using a computer program (Graphpad In State Software, Inc.).

3. Results

The effect of *M. oleifera* on the oxidative stress was clear in some parameters, and the result of five groups were represented in figures (1– 9). In figures the data were presented as five columns; control group, aluminium treated, *M. oleifera* treated, administration of *M. oleifera* for four weeks after administration of aluminium for four weeks and administration of *M. oleifera* along with aluminium for eight weeks.

The activity of SOD shows significance decrease in group 2 only in comparison to control group. The activity of CAT in brain was significantly decreased in groups 2 & 4 and was not significant in groups 3 & 5 in comparison to control group. The activity of GST in brain showed significant decrease in groups 2 and 4 and was not significant in groups 3 & 5 in comparison with control group. The activity of GPx in brain was significantly decreased in groups 2 & 4 in comparison with control. The activity of XO in brain was significantly increased in groups 2 & 4 in comparison with control. The activity of MDA in brain was significantly increased in groups 2 & 4 in comparison with control group. The activity of (MAO) in brain was significantly decreased in groups 2 & 4 while no change in groups 3 & 5 in comparison with control group. The activity of AChE in brain was significantly decreased in groups 2 & 4 while no change in groups 3 & 5 in comparison with control group. The activity of (CK) in brain was significantly increased in groups 2 & 4 while no change in groups 3 & 5 in comparison with control group.

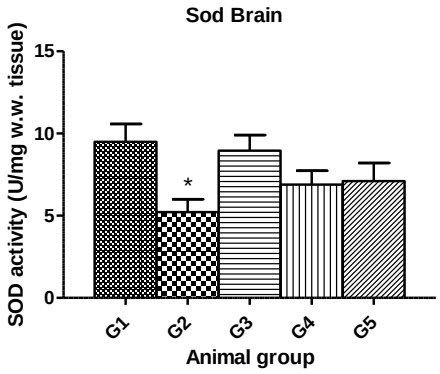

Fig. (1): Brain SOD activity of male rat

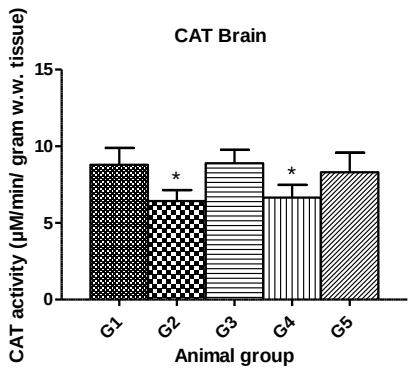

Fig. (2): Brain CAT activity of male rat

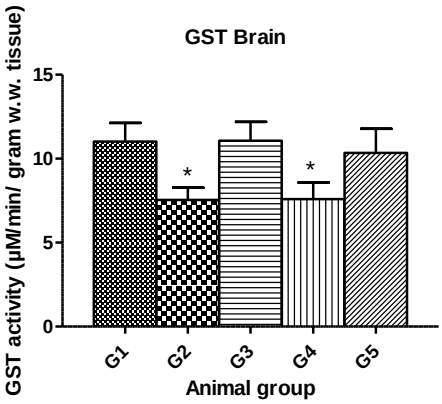

Fig. (3): Brain GST activity of male rat

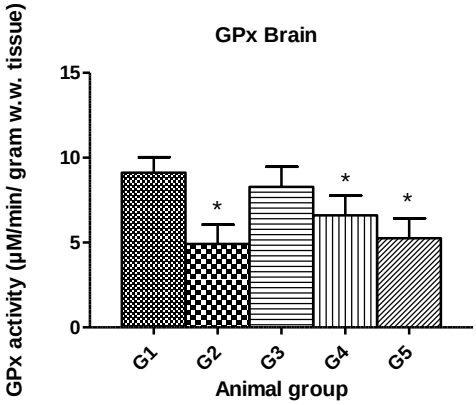

Fig. (4): Brain GPx activity of male rat

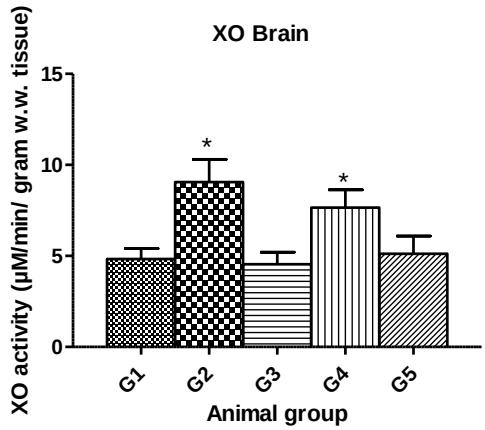

Fig. (5): Brain XO activity of male rat

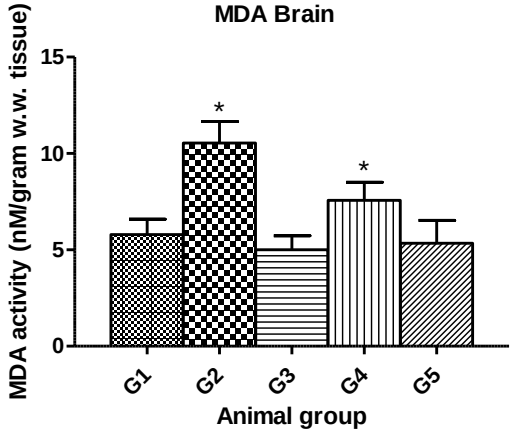

Fig. (6): Brain MDA activity of male rat

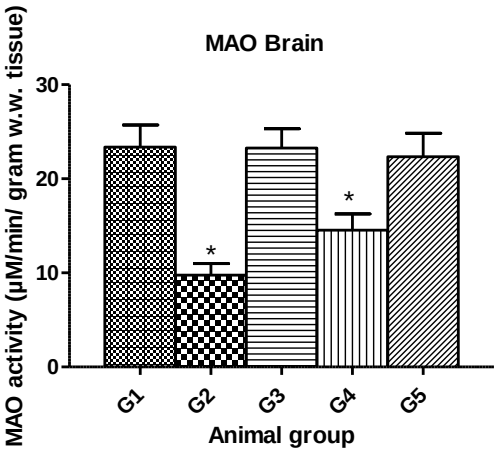

Fig. (7): Brain MAO activity of male rat

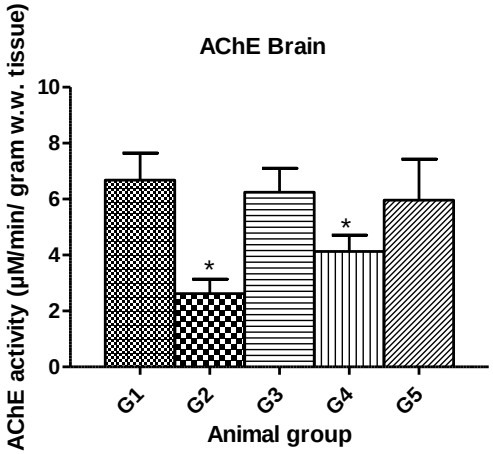

Fig. (8): Brain AChE activity of male rat

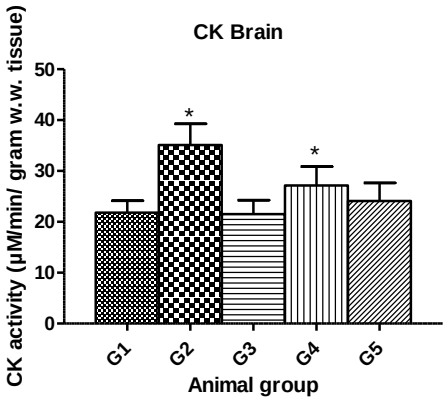

Fig. (9): Brain CK activity of male rat

- Each reading represents means ± SD of 10 rats
- Asterisk indicates a significant decrease in comparison with control at P ≤ 0.05 determined using ANOVA followed by Dennett's test.

4. Discussion

The activity of SOD, CAT, GST and GPx decreased and the level of MDA were significantly increased in brain of aluminium-intoxicated groups (2 & 4) and this agree with other findings of rat brains treated with aluminium (Jongberg *et al.*, 2017). However, (Moumen *et al.*, 1997) found that GPx activity decreased attended with increased SOD activity in rat brain tissue exposed to aluminium. All this agree with (Turgut *et al.*, 2006) who reported that

aluminium exposure can increase MDA level as it interacts with cell membrane directly as aluminium salts. This may accelerate lipid peroxidation in membrane lipids induced by Fe (II) salts. Aluminium ions produce a subtle rearrangement in the membrane structure that facilitated the oxidative action of iron (Hosny *et al.,* 2018). Effects of aluminium-intoxication in various tissues such as brain of different animals were investigated where aluminium increased the rate of lipid peroxidation (Biwas *et al.,* 2017). Investigations have shown the decrease of GPx activity caused by aluminium-intoxication was parallel to the increase of lipid peroxidation rat (Mirshafa *et al.,* 2018, Zaky *et al.,* 2017). MDA activity was increased in aluminium treated group and this agree with (Hassanin *et al.,* 2017) who discussed effect of nutraceuticals in neurotoxicity induced by aluminium oxide in experimental animals as he showed that MDA level increased in aluminium treated group and the level was improved again when groups treated with lecithin and gallic acid.

In the present study the co-supplementation of the herbal antioxidants, *M. oleifera* reduce the damaging effects of aluminium-intoxication, on the activity of these enzymes and lipid peroxidation. *M. oleifera* prevents the inhibition of SOD and CAT activities lipid peroxidation level caused by aluminium. These results agree with other finding (Al-Amoudi, 2018) who studied the ameliorative effect of ginger extract on toxic effects of Lambda-cyhalothrinon in the rat thyroid. Also, this study agrees with (Zakaria *et al.,* 2017), who discussed the activity of SOD, GPx and MDA in cerebral rat tissue after exposure to aluminium; he said that SOD and GPx activity were decreased in group treated with aluminium while the activity of MDA increased in group treated with aluminium. The current results reflect the oxidant properties and ability of *M. oleifera* to scavenging the free radicals.

A total reduction in the aluminium-treated group occurred in AChE activity and it had a marked improvement in the therapeutic group 5 and this agree with (Martinez *et al.,* 2017). They found that aluminium-intoxication for 60 days at low levels1.5 mg/kg b.w/day 8.3 mg/kg b.w/day,or for 42 days at high levels 100 mg/kg b.w/day decreased the enzymatic activity of AChE in hippocampus. On the contrary, Hernández *et al.* (2001) observed an increase on the AChE activity in brain of rats exposed to 10 mg/Kg of Pb-intoxication after 30.0 min, but a strong decrease in this enzyme after 24 & 72 exposure hours. Thus, increase in AChE activity in response to metals seems to be a response to acute short term-exposure while decreases in AChE activity would be expected after more extended exposure.

The activity of CK increased in aluminium intoxicated rats compare to control rats. This may indicate that cell response to the increasing energy needs to cope with aluminium-intoxication. The transfer of phosphate group from creatinine phosphate to ATP in order to generate ATP is done by CK (Srinivas Bharath, 2017, Whiting *et al.,* 2018). CK is found in high concentration in skeletal muscle, myocardium and brain which appear to be sensitive measure of myocardial infraction and muscle diseases, but remains normal in liver disease (Eissa *et al.,* 2017, De la Torre *et al.,* 2000). Abdel-Maksouda *et al.* (2005) reported that there was reduction in the activities of brain enzymes including MAO and AChE in aluminium-intoxicated group compared to control group. MAO catalyzes the oxidative deamination of various primary amines, such as norepinephrine, serotonin, and dopamine. Altogether these findings indicate that long term Al feeding results in inhibition of AchE, and decreased activity of MAO, which could represent the mode of action through which aluminium-intoxicated may further contribute to neurotoxicity.

The enzyme XO considered an important biological source of superoxide radicals (Hegazi *et al.,* 2015). When acting as an NADH oxidase, XO is a generator of superoxide, a powerful reactive oxygen species (ROS). Due to their highly reactive nature, these ROS affect various molecular components of the cell, with excess amounts leading to cell degeneration and death. In mammalian tissues, XO was found predominantly in the liver and intestine (Pierrefiche and Laborit, 1995). 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). Although XO generates ROS, it should note that in vivo, the enzyme exists predominantly as dehydrogenase, reacting with NAD^+. The present increase in the activity of XO may greatly contribute to an increased rate of ROS generation as result to the exposure of aluminium.

M. oleifera administration improve the activity of AChE, MAO, and CK. The potent acute antioxidant effects of the *M. oleifera,* which may be presumably caused by direct scavenging of ROS by the extract constituents. *M. oleifera* reduce the effect of oxidative stress due to the presence of rich combination of antioxidant phytochemicals that have been reported to possess antioxidant, antitumor and anti-inflammatory activities (Patel *et al.,* Jaja-Chimedza *et al.,* 2018).

Finally, it could be concluded that the present study indicated that aluminium-intoxication can affect adversely in vital organs e.g. brain at chronic exposure. The oxidative stress cause changes in brain

parameters. Therefore, it is necessary to create consumer awareness regarding the ill effects of this product and reduce chronic usage of aluminium. Based on our results, we believe that the usage of aluminium in current use is not warranted.

The mechanism of aluminium action as prooxidant may be produced through its interaction with the membranes, subtle changes in the rearrangement of lipids which could attack and facilitate the propagation of lipid peroxidation leads to loss of membrane integrity, decrease its fluidity, disrupt the functioning membrane bound enzymes receptors and ion channels, which leads finally to cell death (Eimil- Fraga *et al.,* 2016). Aluminium-intoxication has increased the lipid peroxidation of platelet membrane in a dose-dependent manner via generation of ROS (Hansen *et al.*, 2017). Aluminium, due to its electronic configuration, does not participate in redox reaction; consequently, its effect is probably due to a direct interaction with cell component, rather than to reactions with oxidative reactive species (Purwaningsih *et al.*, 2015). Recent in vitro studies showed that incubation of aluminium with the human neuronal cells (NT2) produced significant apoptosis even in lower doses due to enhancing the production of cytochrome C, which trigger the cell death cascade process (Kaya *et al.*, 2017). Therefore, one possible mechanism through which aluminium-intoxication produces neurodegeneration is though induction of apoptosis.

Recommendation

Processed and packaged food is a convenient choice for today's busy families and kitchen tools cannot be free of aluminium, so we must find some way to handle the aluminium inside the kitchen and in the packaged food, especially since they have a greater health impact on children compared to adult. Stress has shown that it has a bad effect on vital organs in the body, especially the brain. We can limit these health problems of aluminium intoxication by giving more concern to *M. oleifera*. *Moringa oleifera* also has shown us how effective it on oxidative stress through the result.

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