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Effects of $AlCl_3$ on the Enzymatic Antioxidants of Wister Rats Treated with *Moringa oleifera* Seed Extracts

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Abstract

Determination of Malondialdehyde, MDA in blood plasma or tissue homogenates is one of the useful methods to predict the oxidative stress levels. The current study investigates the ameliorative effects of the seed extracts of *Moringa oleifera* on 35 albino rats induced with $AlCl_3$ toxicity. Biomarkers of oxidative stress (Superoxide Dismutase, SOD; Catalase, CAT; Glutathione Peroxidase, GPx and Malondialdehyde, MDA were assayed. The plant seed extracts were shown to reduce the levels of MDA increased by $AlCl_3$. $AlCl_3$ caused decrease in (glutathione peroxidase) GPx levels as it causes MDA to significantly get elevated. The results showed that GPx decreased from 9.48 ± 0.86 to 6.68 ± 1.73 but upon treatments with 100 mg/kg bw of *M. oleifera,* GPx levels increased to 8.84 ± 0.86 (ethanol) and 8.96 ± 0.86 (aqueous). Increasing the concentrations of the extracts further increased the GPx levels while MDA were reduced.

Keywords: *Moringa oleifera,* Superoxide Dismutase, Catalase, Glutathione Peroxidase, Malondialdehyde

INTRODUCTION

Moringa oleifera is the most widely known and used plant of the Moringaceae-a single genus family with 14 known species. It is called many names in English some of which include Horse radish tree, Radish tree, Drumstick tree, mother's best friend, West Indian bean. The tree is native to the sub-Himalayan regions of north-west India but is now found widely in Africa, South East Asia, the Pacific, Caribbean Islands and South America. *Moringa oleifera* is a fast growing small tree that is adapted to arid, sandy conditions. The plant has long, drumstick shaped pods that contain the seeds.

Many chronic diseases have in their very core a functional and pathophysiological relationship with inflammation and oxidative stress and these effects can be measured in vitro (Fahey, Stephenson, Wade and Talalay, 2013). In their studies Waterman, Cheng, Rojas-Silva, Poulev, Dreifus, Lila and Raskin, (2014) showed the anti-inflammatory effects of isothiocyanate rich extracts of *M. oleifera* in vitro while Ndhlala, Mulaudzi, Ncube, Abdelgadir, du Plooy and Van Staden, (2014) compared the antioxidant variation between *M. oleifera* cultivars. However, it is much more challenging to associate anti-oxidative and anti-inflammatory measurements made in bodily fluids such as blood, urine, or sputum, with clinical symptoms of disease. Kushwaha, Chawla and Kochhar, (2014) conducted a trial on 30 post-menopausal women who were supplemented with 7 g of *M. oleifera* leaf powder daily for 3 months. They reported that there were changes in the antioxidant profile and "oxidative status" upon monitoring blood/serum biomarkers in the women, and there was no report of symptom measurement.

Galuppo, Giacoppo, De Nicola, Iori, Navarra, Lombardo, Bramanti and Mazzon (2014) have reported that the isothiocyanates of *M. oleifera* repress the inflammatory component of experimental autoimmune encephalomyelitis using the rodent models of disease.

Numerous studies examining the potential of aluminum to induce toxic effects in humans or laboratory animals. The nervous system is the most sensitive target of aluminum toxicity and it may induce cognitive deficiency and dementia when it enters the brain (Pandey and Jain, 2013). Aluminium also provokes cardiotoxic, nephrotoxic and hepatotoxic effects (Geyikoglu, Turkez, Bakir and Cicek, 2012). Excessive ingestion of aluminium has been associated with damage of testicular tissues of both humans and animals. Aluminium exposure is suggested cause adverse impact on aspects male reproduction such as to alter the histology of the testis (Buraimoh, Ojo, Hambolu and Adebisi, 2012); deteriorate

spermatogenesis and sperm quality; enhance free radicals and alter antioxidant enzymes (Yousef and Salama, 2009); interrupts the secretion sex hormone (Guo, Lin, Yeh and Hsu, 2005); and biochemical changes in testis and other accessory reproductive organs (Chinoy, Momin and Jhala, 2005).

Oxidative stress is an imbalance between the production of reactive species and antioxidant defense. Keshari, Verma, Kumar and Srivastava, (2015) said oxidative stress is "a disturbance in the pro oxidant-antioxidant balances in favor of the former, leading to serious damage".

Studies of oxidative stress often shifts researcher's attention to its damaging effects on the human body and essential for life and also responsible for the death of a cell. In organisms including human's, reactive oxygen species (ROS) and free radicals are produced during metabolic and immune system function. Molecular oxygen (O_2) has ability to un-pair and leave free radicals which are unstable and highly reactive leads to formation of ROS. When the concentration of ROS beyond a certain limit it is beneficial as biological functions such as phagocytosis, apoptosis, necrosis, and provide protection against pathogens. In oxidation reaction several enzymes (peroxidase) uses hydrogen peroxide (H_2O_2) as a substrate which involving the synthesis of complex organic molecules in the organisms (Keshari and Farooqi, 2014). The body has a defensive mechanism which neutralizes the ROS effect in humans. The principal defensive agents against ROS are antioxidants and endogenous antioxidants (such as Catalase and superoxide dismutase (SOD), small proteins like thioredoxin, glutaredoxin, and molecules such as glutathione etc.). When ROS concentration increases beyond a certain limit it causes damage to the DNA, Proteins, Lipids and carbohydrates, this leads to oxidative stress.

Several studies have shown that oxidative stress is responsible for the development or enhancement of human diseases, such as ulcerative colitis, non-ulcer dyspepsia, Parkinson's disease, Alzheimer's disease, Atherosclerosis, major depression, alcohol induced liver disease, Cancer, diabetic nephropathy, end stage renal disease, Cardiovascular disease, mild cognitive impairment, aging and neural disorders (Keshari, Verma, Kumar and Srivastava, 2015; Kumari, Verma, Rungta, Mitra, Srivastava and Kumar, 2013).

The human body has a balanced system maintained by the DNA, Proteins, Carbohydrates and Lipids. ROS damages to these biomolecules cause disturbances in the metabolic state, growth and development of the cells of organism which leads to serious disease, known as

oxidative stress. Oxidative stress damages nitrogenous base, as well as strand breaks in DNA (e.g. superoxide radical (O_2^-), hydroxyl radical (^-OH) and hydrogen peroxide (H_2O_2). Hydrogen peroxide is produced during oxidative stresses, which are very reactive among the ROS, they cause damages to Proteins, Nucleic acids, Carbohydrates and lipids and leads to oxidative stress (Verma, Chandra, Singh, Singh, Srivastava and Srivastava, 2014).

MATERIALS AND METHODS

Sample Collection and Preparation

Dried seeds of *Moringa oleifera* were collected from a healthy plant from its natural habitat around the Wukari area of Taraba State and was sent to the International Centre for Ethnomedicine and Drug Development in Nsukka, Enugu State where it was identified and authenticated by Ugwu Paschal Ifeanyichukwu (Herbarium Curator) and Alfred Ozioko (taxonomist).

The dried seeds of *Moringa oleifera* were pulverized to powdered specimen using a mortar and pestle.

Sample extraction

A 200g of the powdered seeds was macerated in ethanol and aqueous in the ratio 1:5 for exactly 48hrs. The extracts were filtered out first using a clean white sieving mesh and then using the Watman No. 1 filter paper. The filtrates were concentrated using a rotary evaporator. The concentrated extracts were then transferred to air-tight containers, corked and preserved in the refrigerator at 4 °C until required.

Animals Specimen

35 male albino rats of 100–150 g were purchased from the animal house of the Department of Zoology, University of Nigeria, Nsukka. They were kept in clean cages and maintained under standard laboratory conditions (Temperature 25 ± 5 ^oC, Relative humidity 50 – 60 %, and a 12/12h light/dark cycle) and allowed free access to standard diet and water ad libitum. Animals were allowed to acclimatized for 7 days before each of the experiments. All experiments were conducted in compliance with ethical guide for care and use of laboratory animals of the Department of Zoology, University of Nigeria, Nsukka.

Experimental Design

The rats were randomly divided into seven groups of five animals each (n = 5) as follows:

i. Group 1 Control received normal feed and water + 3 % tween 80 after 1 hour.

ii. Group 2 received 100 mg/kg bw Aluminium chloride + 3 % tween 80 after 1 hour.

iii. Group 3 received 100 mg/kg bw standard drug an hour after administering 100 mg/kg bw of Aluminium chloride.

iv. Group 4 received 100 mg/kg bw ethanol extract of *Moringa oleifera* seeds an hour after administering 100 mg/kg bw of Aluminium chloride.

v. Group 5 received 100 mg/kg bw aqueous extract of *Moringa oleifera* seeds an hour after administering 100 mg/kg bw of Aluminium chloride.

vi. Group 6 received 400 mg/kg bw ethanol extract of *Moringa oleifera* seeds an hour after administering 100 mg/kg bw of Aluminium chloride.

vii. Group 7 received 400 mg/kg bw aqueous extract of *Moringa oleifera* seeds an hour after administering 100 mg/kg bw of Aluminium chloride.

After the experimental period, animals were sacrificed and venous blood was collected by ocular puncture. Blood samples were collected into plain sample tubes containing no anticoagulant for the serum. The blood samples were allowed to clot and the serum was obtained by centrifuging at 3000 rpm for 5 min.

Biochemical Assay

Assay superoxide dismutase (SOD)

Superoxide dismutase (SOD) activity was assayed as formerly described by Fridovich (1989).

The ability of superoxide dismutase to inhibit the autoxidation of adrenaline was the basis of the superoxide dismutase (SOD) assay. Superoxide generated by the xanthine oxidase reaction is known to cause the oxidation of adrenaline to adenochrome. The yield of adenochrome produced per superoxide introduced increased with increasing pH and also with increasing concentration of adrenaline. These led to the proposal that autoxidation of adrenaline proceeds by at least two distinct pathways; one of which is a free radical chain reaction involving superoxide radical and hence could be inhibited by superoxide dismutase (SOD).

Assay of catalase

The activity of catalase was assayed by the method of Sinha ((1972). Dichromate in acetic acid was reduced to chromic acetate, when heated in presence of hydrogen peroxide with the formation of per chromic acid as an unstable intermediate. The chromic acetate formed was measured at 570 nm. Catalase was allowed to split and H_2O_2 was determined by measuring chromic acetate calorimetrically. The requirements are: Phosphate buffer, 0.01 M, pH 7; Hydrogen peroxide, 0.2 M; Potassium dichromate, 5 %; Dichromate acetic acid reagent: Potassium dichromate and glacial acetic acid were mixed in the ratio 1:3; Standard hydrogen peroxide 0.2 M

Determination of the glutathione peroxidase (GPx) activity

The general reaction proformed at 25 ^{0}C and pH 8.0 catalyzed by GPx can be described as follow

$$\text{R-O-OH} + 2\text{GSH} \xrightarrow{\text{GPx}} \text{R-O-H} + \text{GSSG} + H_2O$$

In the assay, Cumenshydroperoxide is used as the peroxide substrate (ROOH), and glutathione reductase (GSSSG-R) and NADPH (β-Notinamide adenine Dinucleotate phosphate, reduced) are included in the reaction mixture the changes in 430 nm due to NADPH oxidation is monitored and is indicative of GPx activity. Cunene hydroperoxide is used to measure the total GPx activity this substrate is suitable for the reaction because it has a low spontonous reaction with GSH, low spontonous hydrolysis and is not metabolized by catalase, one of the other universally present antioxidant enzyme.

Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) using Graph Pad Prism version 5 and Tukey post hoc test. Values of $P< 0.05$ were considered to be significant. The data obtained were expressed in tables and charts.

RESULTS

Qualitative Phytochemical of Ethanol and Aqueous Extract Moringa oleifera seeds

Table 1 shows the presence of reducing sugars and tannins in mild, phenols, alkaloids and steroids in moderate amount while flavonoids and terpenoids were present in abundance in the ethanol extracts of *M. oleifera*. Whereas the aqueous extracts presence of terpenoids and

steroids were mild, reducing sugars, phenols and flavonoids were moderate while saponins and alkaloids are in abundance.

Anthraquinones, carbohydrates and glycosides were absent in both the extracts while saponin was absent in the ethanol extract but abundant in the aqueous extract while tannin also absent in the ethanol extract and mild in the aqueous extract.

Table 1: Qualitative Phytochemical Composition of Ethanol and Aqueous Extract *Moringa oleifera* seeds.

SN	Test	Ethanol	Aqueous
1	Saponin	-	+++/++
2	Anthraquinones	-	-
3	Carbohydrates	-	-
4	Reducing Sugar	**+**	**++**
5	Tannins	+	-
6	Glycosides	-	-
7	Phenols	++	++
8	Alkaloids	++	+++
9	Steroids	++	+
10	Flavonoids	**+++**	++
11	Terpenoids	**++++**	**+**

+mild, ++moderate, +++abundant

Enzymatic antioxidants

Table 2: presents the activity of antioxidant enzymes administered with $AlCl_3$ and extracts of *M. oleifera* seeds. The results showed that CAT and MDA activity was greatly increased when given $AlCl_3$ as compared to the control. SOD and GPx levels decreased from 11.24 ± 0.23 in the control group to 11.14 ± 0.19 and from 9.48 ± 0.86 to 6.68 ± 1.73 respectively.

Administering $AlCl_3$ along with the plant extracts decreased the elevated of CAT and MDA. Increasing the concentrations of the plant extracts causes further decrease in those elevated levels. Meanwhile, the decreased values of SOD and GPx showed significant increase in groups that were administered the plant extracts.

Table 2: Effects of *M. oleifera* seed extract on antioxidant enzymes of $AlCl_3$ treated rats.

	SOD	CAT	GPx	MDA
Group 1	11.24 ± 0.23^{c}	1.07 ± 0.11^{a}	9.48 ± 0.86^{c}	1.35 ± 0.61^{c}
Group 2	11.14 ± 0.19^{a}	2.06 ± 0.17^{e}	6.68 ± 1.73^{a}	2.25 ± 0.48^{d}
Group 3	11.17 ± 0.09^{b}	1.35 ± 0.08^{d}	9.31 ± 1.72^{c}	1.30 ± 0.50
Group 4	11.19 ± 0.16^{b}	1.30 ± 0.17^{d}	8.84 ± 0.86^{b}	1.33 ± 0.27^{c}
Group 5	11.22 ± 0.22^{c}	1.29 ± 0.09^{c}	8.96 ± 0.86^{b}	1.29 ± 0.38^{b}
Group 6	11.37 ± 0.08^{d}	1.19 ± 0.13^{b}	9.31 ± 0.86^{c}	1.16 ± 0.19^{a}
Group 7	11.39 ± 0.06^{d}	1.15 ± 0.20^{a}	9.27 ± 0.86^{c}	1.16 ± 0.17^{a}

Each value represents the mean of 5 rats ± SD.

CAT = Catalase, SOD = Superoxide dismutase, GPx = Glutathione peroxidase, MDA = Malondialdehyde

Group 1: Control, Group 2: 100 mg/kg bw $AlCl_3$, Group 3: 100mg/kg bw $AlCl_3$ + Standard Drug, Group 4: 100mg/kg bw $AlCl_3$ + 100mg/kg bw eth., Group 5: 100mg/kg bw $AlCl_3$ + 100mg/kg bw aq., Group 6: 100mg/kg bw $AlCl_3$ + 400mg/kg bw eth., Group 7: 100mg/kg bw $AlCl_3$ + 400mg/kg bw aq.

DISCUSSION

Phytochemicals of Moringa oleifera

A screening for phytochemicals or a qualitative analysis is used to reveal the chemical constituents or the secondary metabolites of plant extracts. In this study, preliminary phytochemical screening for seed extracts of *M. oleifera* revealed that flavonoids and terpenoids were abundantly present in the ethanol extracts as shown in Table 1 above. In the same ethanol extracts, phenols, alkaloids and steroids were moderately present whereas, reducing sugars and tannins were mild. Saponins which were not present in the ethanol extracts were now found to be abundant in the aqueous extracts. Alkaloids were also abundant. Reducing sugars and phenols were now seen to be present in moderate concentrations. Steroids and terpenoids were in mild concentrations. In their studies Fahal, Rani, Aklakur, Chanu and Saharan (2018) reported the presence of alkaloids, flavonoids, saponins, sterols and tannin in the ethanol and aqueous extracts of *M. oleifera* pods.

Several studies have shown that phytochemicals are supportive in the prevention and healing of infections and illnesses such as stimulating the body's immune system against various diseases causing agents like bacteria, fungi, viruses, etc.

Flavonoids have been said to help in treatment of inflammation and boosting the immune system while alkaloids, tannins, saponins and phenols have antimicrobial activity and as such essential in treatments of malaria. This suggest the use of this plant in the traditional treatment of several diseases (Khan, Suleman, Baqi, Ayub and Ayub, 2018).

Effects of $AlCl_3$ on enzymatic antioxidants

Lipid peroxidation is usually used as an indicator of oxidative stress in different tissues (Khafaga and Bayad, 2016). Malondialdehyde (MDA) represents the indicative end product of lipid peroxidation process in various tissues or biological fluids (Khafaga, 2017)

Table 1 shows that there were increase in the levels of MDA when animals were ex\posed to $AlCl_3$. Aluminum exposure can increase MDA level as it interacts with cell membrane directly as aluminum salts (Turgut, Enli, Kaptanoğlu, Turgut and Genç, 2006). This may accelerate lipid peroxidation in membrane lipids induced by Fe (II) salts. Aluminum ions produce a subtle rearrangement in the membrane structure that facilitates the oxidative action of iron (Hosny, Sawie, Elhadidy and Khadrawy, 2018). Investigations have shown the decrease of GPx activity caused by aluminum-intoxication was parallel to the increase of lipid peroxidation rat (Zaky, Bassiouny, Farghaly and Elsabaa, 2017; Mirshafa, Nazari, Jahani and Shaki, 2018). The report of this study also agrees with such investigations as because the levels of GPx decreased from 9.48 ± 0.86 to 6.68 ± 1.73 although treatments with 100 mg/kg bw of *M. oleifera* increased the GPx levels to 8.84 ± 0.86 (ethanol) and 8.96 ± 0.86 (aqueous). Increasing the concentartions of the extracts further increased the GPx levels.

SOD is an important antioxidant enzyme; it is considered as the first enzymatic defense against the superoxide anion. SOD is responsible for catalyzing the ROS binding with water to generate H_2O_2.

Following that, the breakdown of H_2O_2 to water and oxygen is occurred via CAT to protect cells from the damaging effect of H_2O_2 and the hydroxyl radicals. These events may introduce an accepted explanation for the reduction in SOD and CAT level reported in the present study after exposure to $AlCl_3$.

CONCLUSION

This studies demonstrates the effectiveness of *Moringa oleifera* seeds against oxidative stress caused by $AlCl_3$ intoxication of Wistar rats. The low mortality rate observed during the experiment suggest the safety and efficacy of this plant extract in the system.

The hypotheses of this study is stated below

H1: $AlCl_3$ has effects on the enzymatic antioxidants albino Wister rats treated with *Moringa oleifera* seed extracts.

Recommendation

Further studies in regards to the effects of *Moringa oleifera* seed extracts should be considered in novel drug development. The plant should be considered a traditional drug for health remedy. The extract is a promising source for therapeutic agent that can be used in combating infectious diseases caused by drug-resistant bacteria, since this study shows that the extract exhibited significant improvements in the activities of serum electrolytes as against albino Wister rats induced with $AlCl_3$ toxicity, further studies should be carried out for the isolation and identification of individual bioactive compounds which are responsible for this therapeutic activity and the investigation of their mechanism(s) of action.

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