Comparative Evaluation of Oxidative Stress Indices in Albino Rats Administered Aqueous, Ethanol and Methanol Extracts of *Moringa oleifera* leaves and Seeds Locally Grown in Abakaliki, Nigeria

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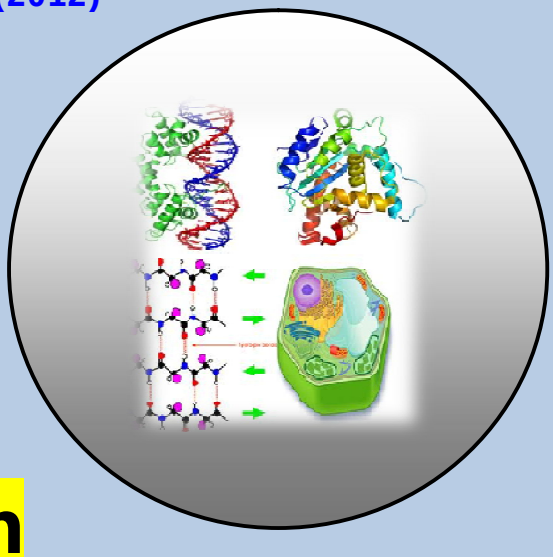

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Comparative Evaluation of Oxidative Stress Indices in Albino Rats Administered Aqueous, Ethanol and Methanol Extracts of *Moringa oleifera* leaves and Seeds Locally Grown in Abakaliki, Nigeria

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ABSTRACT

The effect of aqueous, ethanol and methanol extracts of dried leaf and seed of Moringa oleifera on reduced glutathione (GSH), catalase, superoxide dismutase (SOD) and malondialdehyde (MDA) levels were evaluated using 52 albino rats. The rats were randomly assigned to four experimental groups marked A, B, C and D. Group A rats (n=4) received only water and feed and served as control while B, C and D received aqueous, ethanol and methanol extracts of Moringa oleifera leaf and seed. Test groups B, C and D were sub-grouped into four groups with four rats each corresponding to B1, B2, B3, B4, C1, C2, C3, C4 and D1, D2, D3 and D4 respectively. The test groups were administered daily with aqueous, ethanol and methanol extracts of M. oleifera leaf and seed at the graded doses of 100, 200, 400 and 800 mg/kg body weight to the rats respectively for 21 days. The percentage yield of the aqueous, ethanol and methanol extracts of M. oleifera leaf and seed were 20.44, 22.07 and 22.38 % respectively. The results obtained showed that there was no significance difference ($p>0.05$) in the mean body weight of the rats among the administered groups. The administration of the aqueous, ethanol and methanol extracts of Moringa oleifera leaf and seed at 100, 200,400, and 800 mg/kg body weight significantly decreased($p<0.05$) the level of malondialdehyde (MDA) in a dose dependent manner. The same extracts at various doses significantly ($P<0.05$) increased the level of reduced glutathione (GSH), catalase and superoxide dismutase (SOD) in rats in a dose dependent manner. The result also revealed that there was no significant ($P>0.05$) difference among the rats fed seed and leaf extracts in the above biochemical parameters evaluated. The result also revealed that no significant ($p>0.05$) difference was observed in the levels of the oxidative stress indices in the rats within the administration period at all doses using the various solvent extracts. The results also revealed that locally grown M. oleifera leaves and seed could be useful in the management of oxidative stress.

Key Words: Moringa oleifera, Aqueous, Ethanol, Methanol, catalase, superoxide dismutase, reduced glutathione, malondialdehyde and Albino rat.

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INTRODUCTION

There are several sources from which the reactive oxygen species are generated. Most reactive oxygen species come from the endogenous sources as by-products of normal and essential metabolic reactions, such as energy generation from mitochondria or the detoxification reactions involving the liver cytochrome p-450 enzyme system. Other enzymes capable of producing superoxide are xanthine oxidase, and NADPH oxidase (Norbert, 2001). Metals such as iron, copper, chromium and cobalt are capable of redox cycling. This is a process in which a single electron may be accepted or donated by the metal (Norbert, 2001). Most enzymes that produce reactive oxygen species contain one of these metals mentioned above. The presence of such transition metals in biological system in an un complexed forms (not in protein or other protective metal complex) can significantly increase the level of oxidative stress (Chance *et al.,* 1995). For instance, the hydroxyl radical produced from reduced iron and hydrogen peroxide can lead to modifications of amino acids and carbohydrates and can initiate lipid peroxidation and oxidize bases in nucleic acid (Pavojic *et al.,* 2001). Certain organic compounds in addition to metal redox catalysts can also produce reactive oxygen species. One of the most important classes of these is the quinones. Quinones can undergo redox cycle with their conjugate semi-quinones and hydroquinones, in some cases catalyzing the production of superoxide from di-oxygen or hydrogen peroxide from superoxide (Portaki *et al.,* 2000). Exogenous sources include: cigarette smoke, environmental pollutants such as emission from automobiles and industries, excessive consumption of alcohol, asbestos, ionizing radiation, bacterial, fungal or viral infections, increased exposure to sunlight and excessive exercise (Giordano, 2005). Oxidative stress is a reflection of an imbalance between the rate of production of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting the damage (Valko *et al*., 2007). As the body respires it constantly reacts with oxygen and the cells produce energy, as a consequence of this activity, highly reactive molecules known as free radicals are produced within the cells and oxidative stress occurs (Schafer *et al.,* 2001). Disturbances in the normal redox state of cells can cause toxic effects through the propagation of peroxides and free radicals that damage all components of the cell, including proteins, lipids and DNA (Diego *et al,* 2009). In human's oxidative stress is thought to be implicated in the development of cancer, Parkinson's disease, Alzheimer's disease, atherosclerosis, heart failure, myocardial infarction, sickle cell disease, autism, and chronic fatigue syndrome (Gwen *et al*., 2005 and Halliwel, 2007). The oxidative stress markers assayed for in this research include, superoxide dismutase (SOD), reduced glutathione, peroxidase, catalase and lipid peroxidation. These markers show an index of the level of oxidative stress in a living system. Superoxide dismutases are enzymes that catalyze the dismutation of superoxide into hydrogen peroxide and water and are an important antioxidant defence in nearly all cells exposed to oxygen (McCord *et al.,* 1998). Catalase is a common enzyme found in nearly all living organisms exposed to oxygen, ranging from vegetables to animals.

It catalyzes the decomposition of hydrogen peroxide to water and oxygen, thus, protecting the cell from oxidative damage. (Chelikani *et al.,* 2004). Glutathione on the other hand serves as an antioxidant by serving as an electron donor when reacting with disulfide bonds formed within cytoplasmic proteins to cysteines (Couto *et al.,* 2013). Peroxidases convert hydrogen peroxide to less reactive species like alcohols and are known to help increase plants defenses against pathogens. Finally, lipid peroxidation is an indicator of the level of oxidative damage, a process in which free radicals "steal" electrons from the lipids in cell membrane (McCord *et al.,* 1998). An increase in the activities of the first four markers indicates a good index of oxidative stress, whereas, an increase in lipid peroxidation shows increase of oxidative damage to cells (Daniela *et al.,* 2001). *Moringa oleifera* is commonly known as drumstick-tree or horse radish tree. It is used as vegetable and also in Indian folk medicine for the treatment of various illnesses (Ranjan *et al.*, 2009). *Moringa oleifera* is a small graceful tree with sparse foliage often planted in compounds or used in fencing in Nigeria. It resembles a leguminous species at a distance especially when flowering. The methanolic leaf extract of *Moringa oleifera* has more chemical constituents than the seed with 9-octadecenoic acid (20.8%) as the highest in the leaf and oleic acid (84%) in the seed. These relatively diverse chemical constituents may be responsible for the medicinal properties of *Moringa oleifera* leaves and seeds (Aja *et al*., 2014).The study therefore evaluates the comparative effect on antioxidant activities of *Moringa oleifera* leaves and seeds extracts grown in Abakaliki, Nigeria in albino rats.

MATERIAL AND METHODS

Collection and Preparation of *Moringa oleifera Leaf and Seed Extracts*

The leaves and seeds of *Moringa oleifera* were collected from Abakaliki local government area of Ebonyi State, Nigeria, and identified by taxonomist in the Department of Applied Biology, Ebonyi State University, Abakaliki, Nigeria. A part was also deposited in the hebarium for reference purposes. The leaves and seeds of *M. oleifera* were washed thoroughly under running tap water, shade dried and pulverized, using a grinding machine. Exactly 100g of powdered seeds were soaked in 500, 500 and 500 ml of distilled water, ethanol and methanol respectively for 24hours. The mixtures were sieved and the solvents removed with rotary evaporator to obtain the crude extracts (Oluduro and Aderiye, 2009).

Experimental Animals

A total of 52 albino rats weighing (120-240 g) were used for the study. They were purchased from the Pharmacy Department University of Nigeria, Nsukka, Nigeria and acclimatized (for 7 days) and maintained at normal room temperature in the Experimental Animal House of the Faculty of Biological Sciences, Ebonyi State University, Abakaliki, Nigeria. They were housed in stainless rat's cages and allowed access to water and food *ad- libitum.* At the start of the experiment, all the animals were weighed and subsequently at week intervals. The rats were randomly assigned to three experimental groups marked as groups A, B, C and D. Group A with four rats received only water and food and served as control while B, C and D received aqueous, ethanolic and methanolic extracts of *Moringa oleifera* seeds.

Groups B, C and D were sub-grouped into four groups with four rats each. The sub-groups were administered daily with aqueous, ethanolic and methanolic extracts of *M. oleifera* seeds at the graded doses of 100, 200, 400 and 800 mg/kg body weight to the rats respectively for 21 days. The administration was done through the mouth with diabetic syringe.

Experimental Design

In this experiment, a total of 52 albino rats were used. The rats were grouped into group A with 4 rats which served as control and received no extract. The test groups B, C and D were subdivided into B1, B2, B3, B4, C1,C2, C3, C4, D1, D2, D3 and D4 and were administered graded doses of 100, 200, 400 and 800 mg//kg body weight of the extracts respectively. The animals in the test groups were treated with varying concentration of aqueous, ethanol and methanol extracts of *M. oleifera* seeds. For 21 days. **Group A:** Received no extract. Group B1: Albino rats received aqueous extract of *M. oleifera leaves and* seeds of 100mg/kg body weight daily for 21 days. Group B2: Albino rats received aqueous extract of *M. oleifera leaves and* seeds of 200mg/kg body weight daily for 21 days. Group B3: Albino rats received aqueous extract of *M. oleifera leaves and seeds* of 400mg/kg body weight daily for 21 days. Group B4: Albino rats received aqueous extract of *M. oleifera leaves and* seeds of 800mg/kg body weight daily for 21 days. Group C1: Rats received ethanol extract of *M. oleifera leaves and* seeds of 100mg/kg body weight daily for 21 days. Group C2: Rats received ethanol extract of *M. oleifera leaves and* seeds of 200mg/kg body weight daily for 21 days. Group C3: Rats received ethanol extract of *M. oleifera leaves and* seeds of 400mg/kg body weight daily for 21 days. Group C4: Rats received ethanol extract of *M. oleifera leaves and* seeds of 800mg/kg body weight daily for 21 days. Group D1: Rats received methanol extract of *M. oleifera leaves and* seeds of 100mg/kg body weight daily for 21 days. Group D2: Rats received methanol extract of *M. oleifera leaves and* seeds of 200mg/kg body weight daily for 21 days. Group D3: Rats received methanol extract of *M. oleifera l*eaves and seeds of 400mg/kg body weight daily for 21 days. Group D4: Rats received methanol extract of *M. oleifera* leaves *and* seeds of 800mg/kg body weight daily for 21 days. After the treatment period (21 days), the animals of all the groups were sacrificed. The rats were dissected and 5 ml of whole blood drawn through cardiac puncture. The blood was dispensed into centrifuge tubes and centrifuged at 5000 rpm for 10 minutes. After centrifugation, the serum was then separated from the blood cells and used for assay of serum reduce glutathione, catalase, superoxide dismutase and malonyaldehyde levels were determined using the method described by Oyedemi *et al*.(2010). Statistical analysis: Data obtained were subjected to a one way analysis of variance ANOVA using the General Liner Model procedure of SAS (version 6.04) (SAS Institute, 1994). Comparison of significant treatment means was by least significance differences (LSD) as outlined by Obi (2002).

RESULTS

The administration of the aqueous, ethanol and methanol extracts of *Moringa oleifera* leaves or seeds at 100, 200,400, and 800 mg/kg body weight to albino rats significantly ($P<0.05$) increased the level of reduced glutathione (GSH), % inhibition of superoxide dismutase (SOD)

and catalase (CAT) level in rats in the doses dependent manner while the administration of the same extracts significantly (P<0.05) decreased the level of malondialdehyde (MDA) in a doses dependent manner. The results showed that there was no significant (P>0.05) difference among the rats fed seed or leaf extracts (figure 1-8).

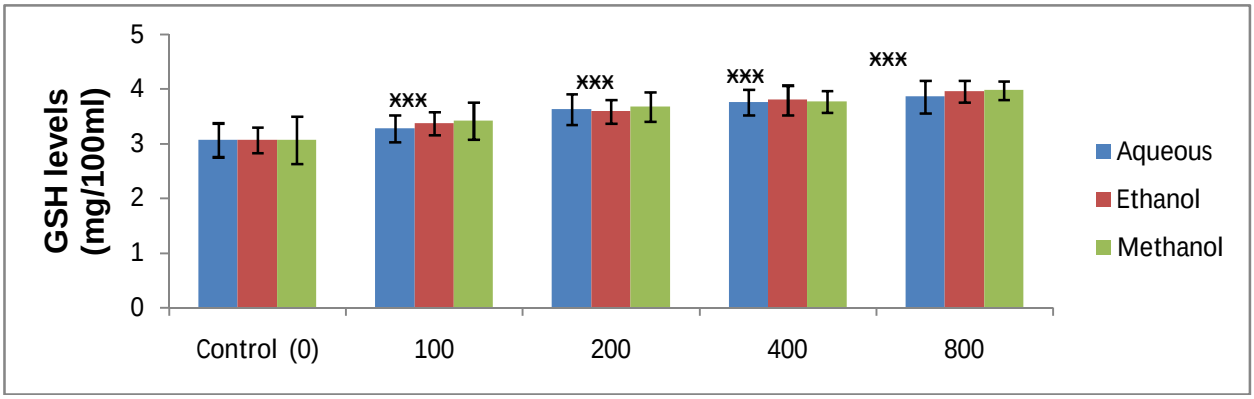

Figure 1. GSH Level in Albino Rats Administered aqueous, ethanol and methanol extracts of *M. oleifera* Leaf.

Data are shown as mean ± S.D (n=8). Mean values in bars with (⁎) have significant differences (P<0.05) when compared with the control.

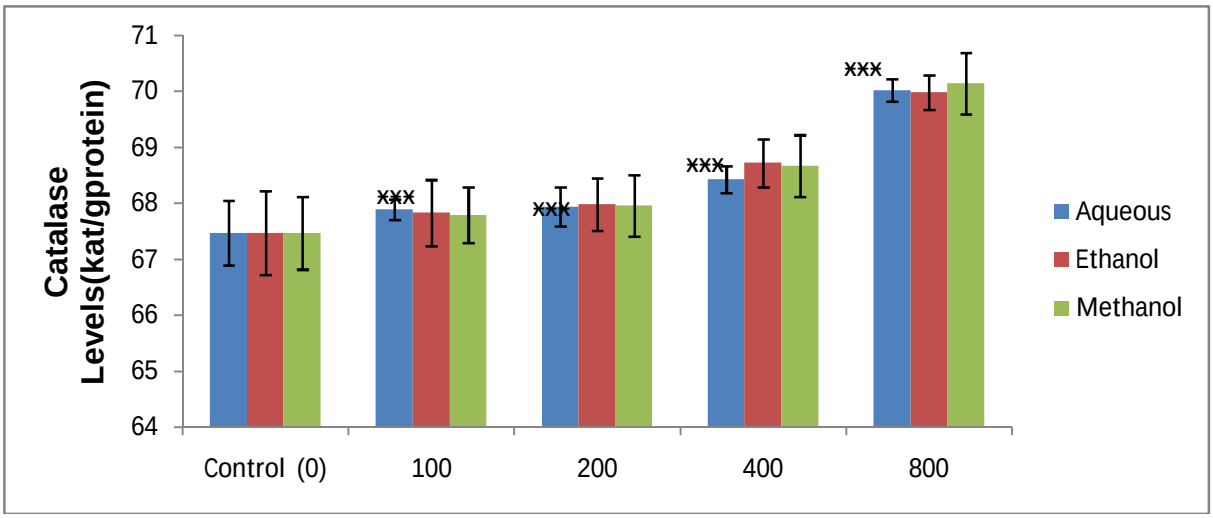

Figure 2. Catalase Levels in the Blood of Albino Rats Administered aqueous, ethanol and methanol extracts of *M. oleifera* Leaf.

Data are shown as mean ± S.D (n=8). Mean values in bars with (⁎) have significant differences (P<0.05) when compared with the control.

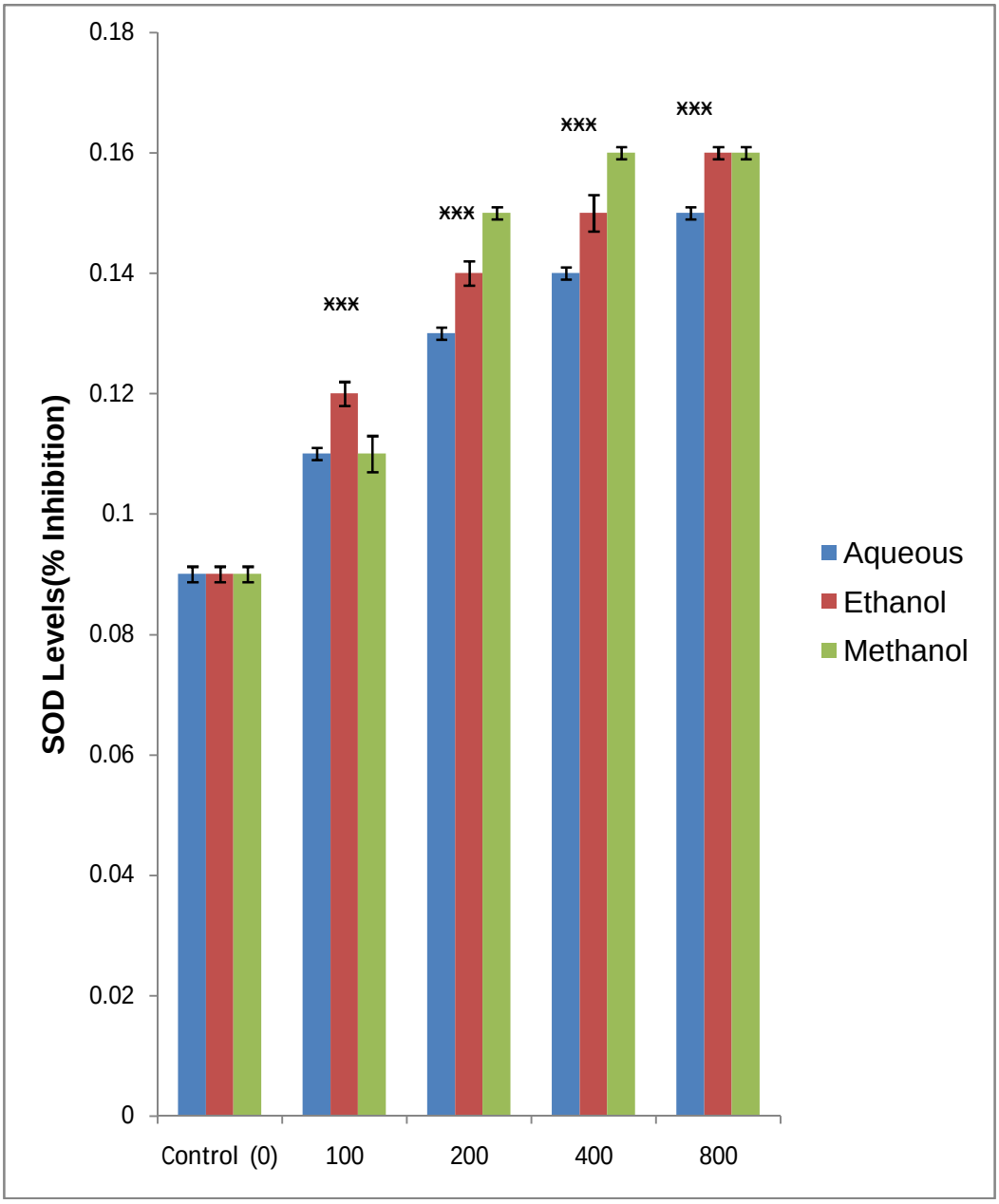

Figure 3. Serum SOD Levels in Albino Rats Administered aqueous, ethanol and methanol extracts of *M. oleifera* Leaf.

Data are shown as mean ± S.D (n=8). Mean values in bars with (⁎) have significant differences (P<0.05) when compared with the control.

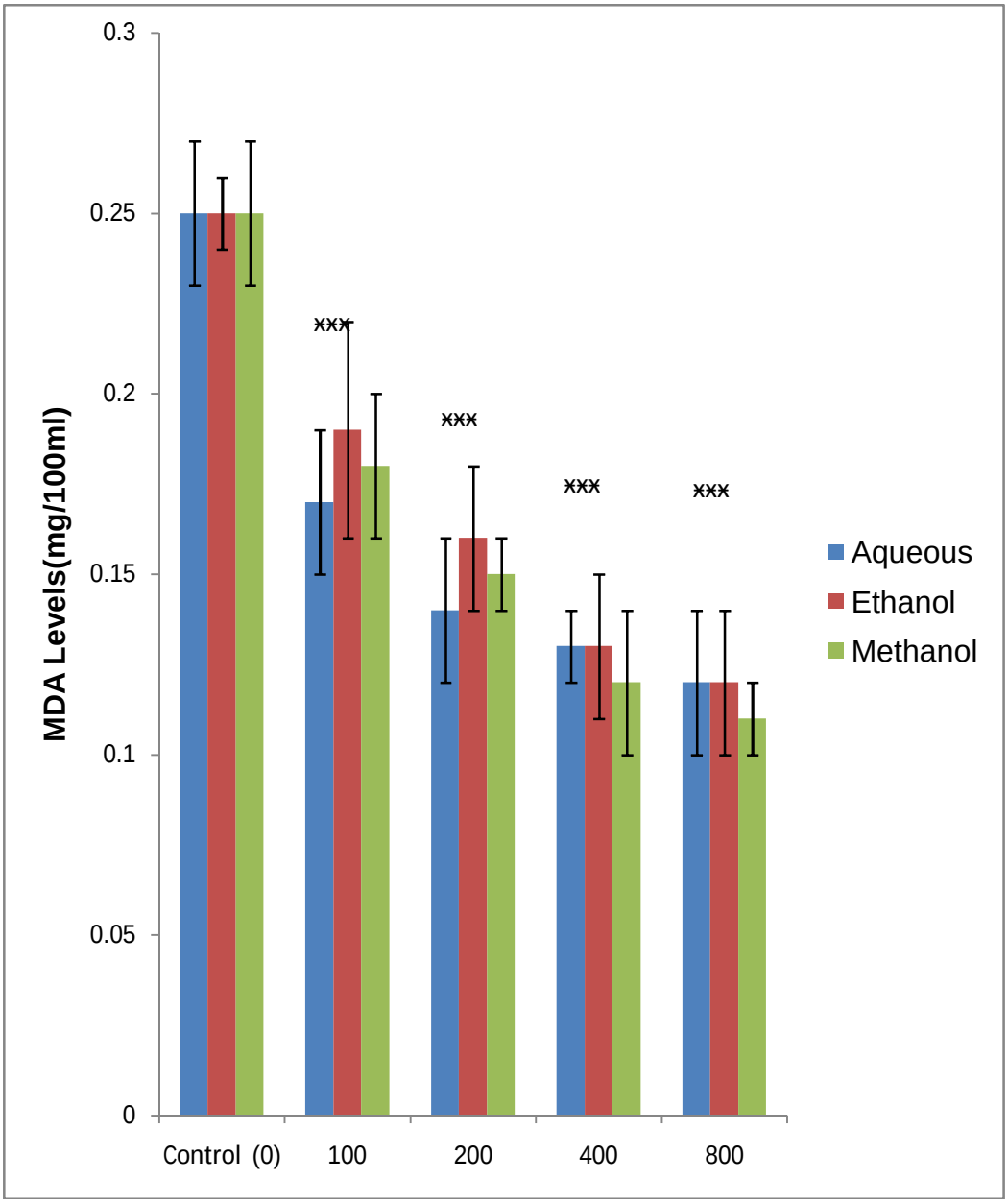

Figure 4. Serum MDA Levels in Albino Rats administered aqueous, ethanol and methanol extracts of *M. oleifera* Leaf.

Data are shown as mean ± S.D (n=8). Mean values in bars with (⁎) have significant differences (P<0.05) when compared with the control.

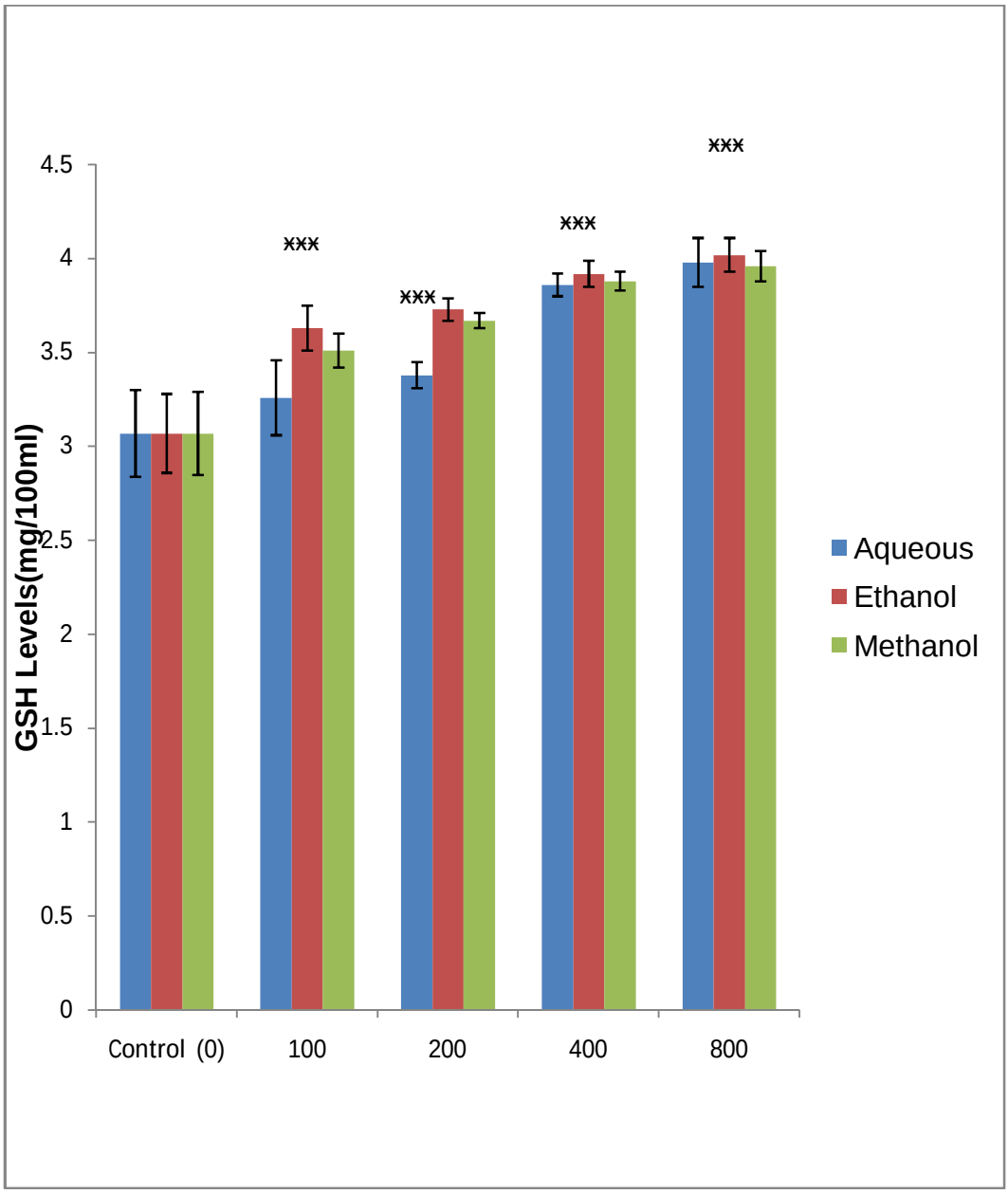

Figure 5. Blood GSH Levels in Albino Rats Administered aqueous, ethanol and methanol extracts of *M. oleifera* Seed.

Data are shown as mean ± S.D (n=8). Mean values in bars with (⁎) have significant differences (P<0.05) when compared with the control.

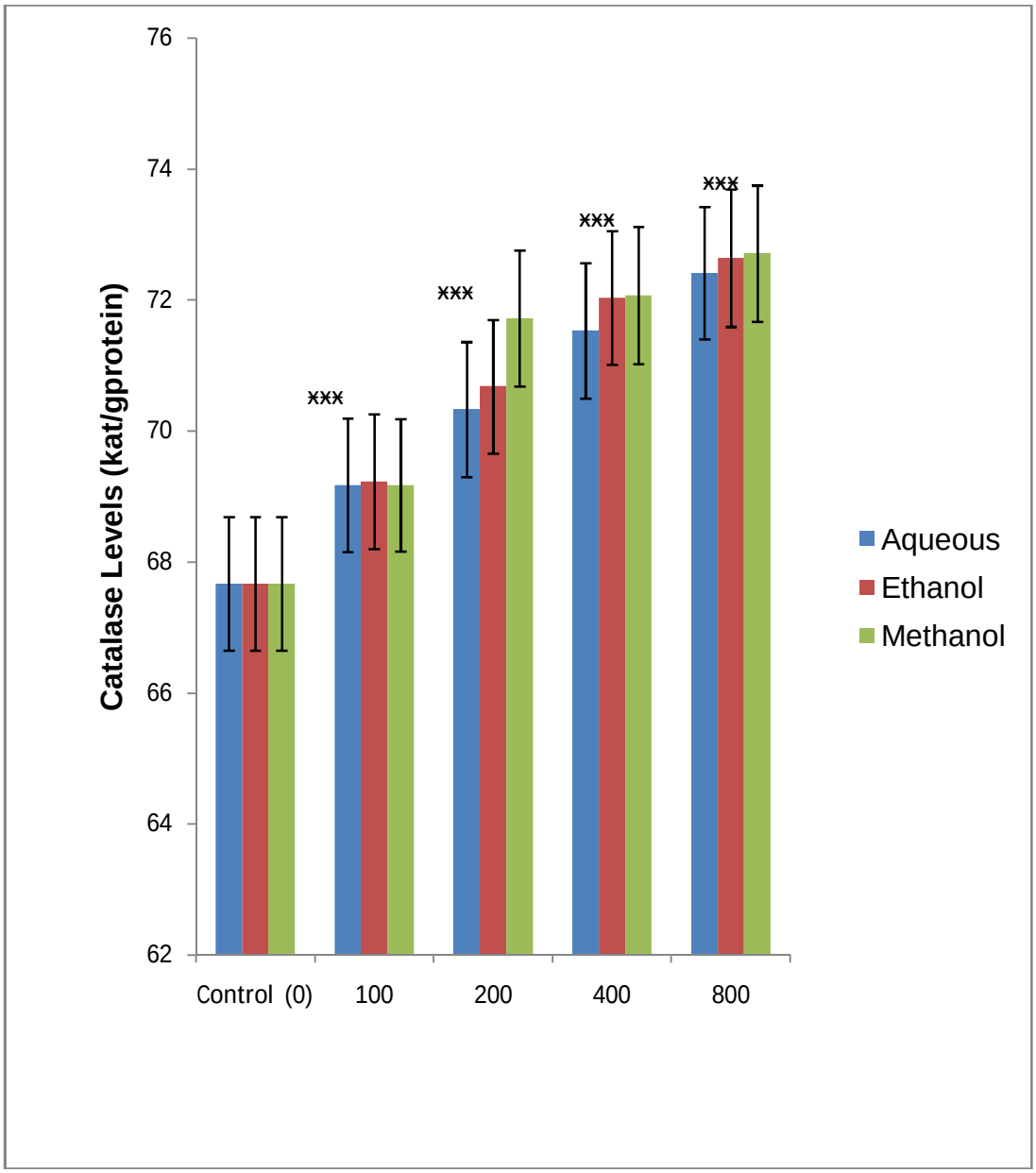

Figure 6. Catalase Levels in the Blood of Albino Rats administered aqueous, ethanol and methanol extracts of *M. oleifera* Seed.

Data are shown as mean ± S.D (n=8). Mean values in bars with (⁎) have significant differences (P<0.05) when compared with the control.

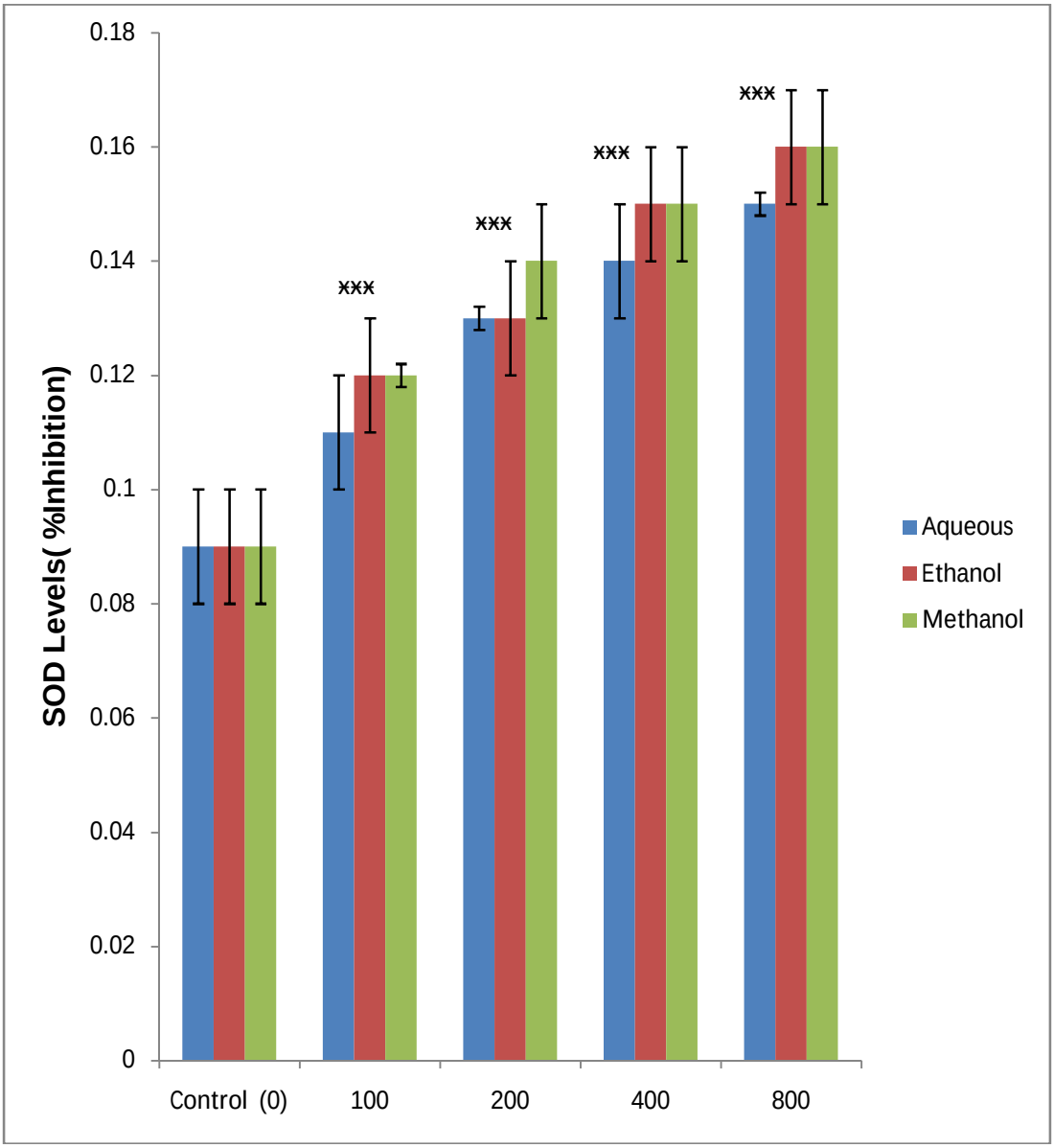

Figure 7. Serum SOD Levels in Albino Rats administered aqueous, ethanol and methanol extracts of *M. oleifera* Seed.

Data are shown as mean ± S.D (n=8). Mean values in bars with (⁎) have significant differences (P<0.05) when compared with the control.

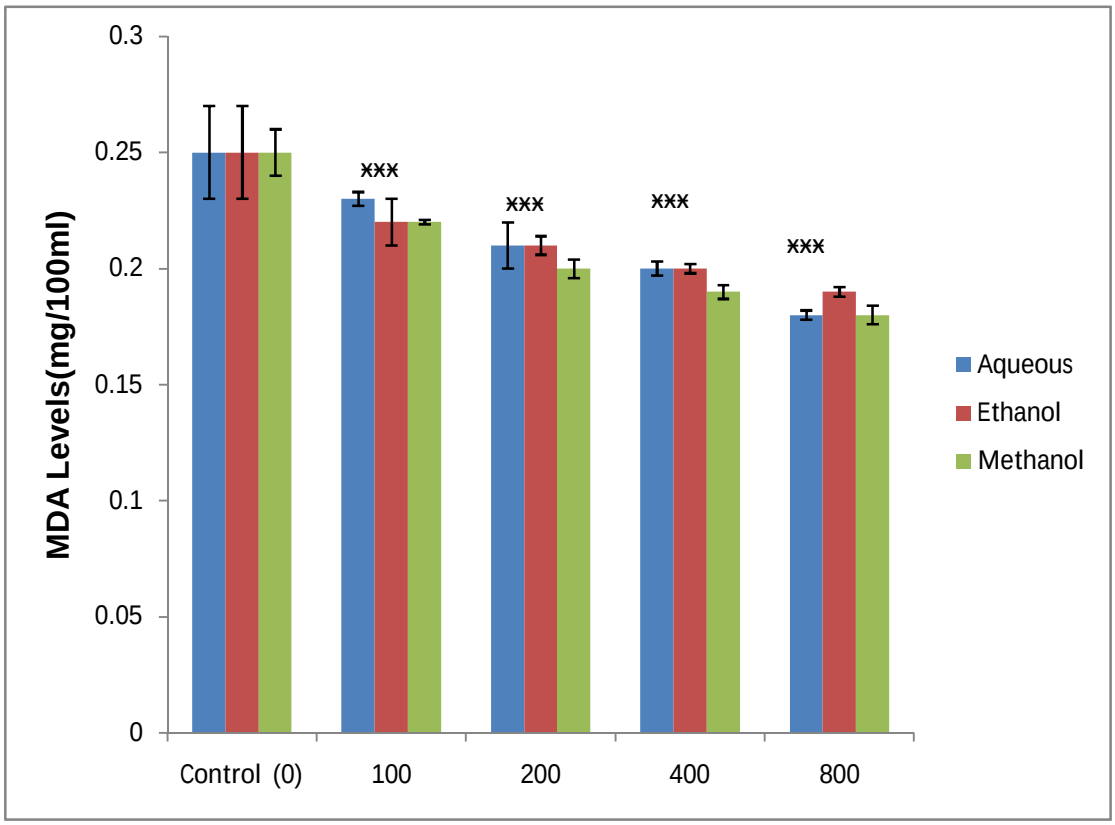

Figure 8. Serum MDA Level in Albino Rats administered aqueous, ethanol and methanol extracts of *M. oleifera* Seed.

Data are shown as mean ± S.D (n=8). Mean values in bars with (⁎) have significant differences (P<0.05) when compared with the control.

DISCUSSION

Moringa oleifera seed and leaf extracts significantly increased (P <0.05) serum superoxide dismutase (SOD), glutathione (GSH) and catalase (CAT) levels at all the doses. Under oxidative conditions, the concentration of the glutathione can be considerably diminished through conjugation to xenobiotics, and by secretion of both the glutathione conjugates and glutathione disulfide from the affected cells (Sies, 1994). Superoxide dismutase has been reported as one of the most important enzymes in the enzymatic antioxidant defense system (Oyedemi *et al.,* 2010). It removes superoxide anion by converting it to hydrogen peroxide, and thus diminishing the toxic effect caused by this radical. However, an increase in the percentage inhibition of SOD after plant extract administration implies an efficient protective mechanism of this plant.

Superoxide dismutase plays an important role in catalyzing the dismutation of superoxide radicals. SOD enzymes work in conjunction with H_2O_2 removing enzymes, such as catalase and glutathione peroxidase. These antioxidant enzymes depend on various essential trace elements and prosthetic groups for proper molecular organization and enzymatic action. Increase in SOD activity should accelerate the removal of the reactive oxygen species. Catalase, whose activity has also been augmented by *Moringa oleifera* extracts, helps in removing the hydrogen peroxide produced by the action of SOD. Induced SOD activity along with that of catalase explains the decrease in lipid peroxidation, which is an indicator of oxidative stress that persists in the cell. The decreased lipid peroxidation in the present study is in correlation with the induction of antioxidant enzymes above basal level by the *Moringa oleifera* extract. Catalase is another antioxidant enzyme widely distributed in the animal tissues. It decomposes to hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals (Oyedemi *et al.*, 2010). The increase in the levels of antioxidant profiles that is SOD, GSH and catalase by *Moringa oleifera* leaves and seeds extracts may be attributed to have biological significance in eliminating reactive free radicals that may affect the normal functioning of cells. Flavonoids can directly react with superoxide anions and lipid peroxyl radical and consequently inhibit or break the chain of lipid peroxidation. This radical scavenging activity of extracts could be related to the antioxidant nature of polyphenols or flavonoids, thus contributing to their electron/hydrogen donating ability. Any natural compound with anti- oxidant properties may help in maintaining health when continuously taken as components of dietary foods, spices or drugs. The increase in the levels of antioxidant profiles, that is, SOD, GSH and catalase by *Moringa oleifera* leaves and seeds extracts may be have biological significance in eliminating reactive free radicals that may affect the normal functioning of cells. The degree of lipid peroxidation is often used as an indicator of ROS mediated damage and the concentration of Malonaldehyde (MDA) in blood and tissues are generally used as biomarker of lipid peroxidation (El-Bahr, 2013)

CONCLUSION

The evaluation of oxidative stress indices with *Moringa oleifera* leaves and seeds extracts have shown that the plant parts could be of great importance for controlling the ROS- mediated pathogenesis; hence the use of *Moringa oleifera* in the management of cardiovascular problems should be encouraged.

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