African Journal of Pharmaceutical Research & Development

Vol. 12 No.1;pp. 026-037(2020)

METHANOL EXTRACT OF *Acanthus Montanus* (ACANTHACEAE) LEAVES AMELIORATES OXIDATIVE STRESS AND IMPROVES HAEMATOLOGICAL INDICES IN RATS

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ABSTRACT

This study evaluated effects of methanol extract of *Acanthus montanus* leaves on oxidative stress markers and haematological indices in acetaminophen oxidative stress induced in rats. Forty-five male Wistar rats were divided into 9 groups of 5 rats and used for this study. Group 1 served as the normal control, group 2 and 3 served as negative and positive controls respectively. Group 4 and 5 were not oxidative stress induced but received 200 and 500 mg/kg of the methanol extract of *A. montanus* leaves. Group 6 – 7 were pre-treated with 200 and 500 mg/kg body of the methanol extract for 7 days before oxidative stress induction and treated with the extract for additional 7 days. Group 8 and 9 were oxidative stress induced and after 24 h treated with 200 and 500 mg/kg of the methanol extract respectively. The oxidative stress induction caused significant ($P < 0.05$) reductions in the activities of antioxidant enzymes (glutathione peroxidase, superoxide dismutase and catalase) and significant ($P < 0.05$) increase in the malondialdehyde concentrations relative to the normal control. These indicated that the rats suffered oxidative stress and lipid peroxidation. Significant ($P < 0.05$) reduction in the red blood cell count, white blood cell count, packed cell volume and haemoglobin concentrations were observed in oxidative stress induced rats, which predisposed them to anaemia. Treatment with the extract significantly ($P < 0.05$) restored the activities of the antioxidant enzymes, reversed the elevated levels of malondialdehyde and decreased levels of haematological indices to normal levels relative the negative control. The findings of this study show that the methanol extract of *A. montanus* leaves could ameliorate the effects of oxidative stress in the body, including prevention of lipid peroxidation and replenishing haematological components.

KEYWORDS: ***Acanthus montanus*, acetaminophen, oxidative stress, antioxidant enzymes, lipid peroxidation, haematology.**

INTRODUCTION

Oxidative stress is a condition that results when there is abnormally high levels of oxidants or free radicals and relatively low levels of enzymatic and non-enzymatic antioxidant systems in the body to scavenge and prevent oxidative damage from free radical attack [1]. Free radicals, generally are molecules with unpaired electron found in biological systems and are commonly referred to as reactive oxygen or nitrogen species (ROS or RNS) that are highly reactive including superoxide radicals, singlet oxygen and nitric oxide radicals among others [2, 3]. Well-known sources of free radicals in the body include leakage from mitochondrial electron transfer

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chain, reactions of xanthine oxidase, cytochrome P450 enzymes, lipoxygenase and peroxisomes [4]. Free radicals can attack and damage biomolecules such as proteins, lipids and nucleic acids resulting in various disease conditions that can negatively impact the quality of life [5, 6]. When free radicals overwhelm the antioxidant systems, oxidative damage occurs and may lead to degenerative diseases, inflammation, mutation and carcinogenesis if not well treated [7]. Outside oxidative damage, free radicals play critical role innate immune system, regulation cell proliferation; wound healing, angiogenesis, neuronal signalling, and thyroid hormone metabolism [8]. Molecules with ability to donate electrons to quench or inhibit propagation of free radical actions can slow or prevent oxidative damage to DNA, tissues, proteins, lipids and other vital organelles, and are therefore referred to as antioxidant molecules [9].

Acetaminophen (N-acetyl-p-aminophenol imine) is a well-known analgesic drug used as pain reliever and antipyretic and is among the most commonly abused drugs in our societies due to self-prescription and lack of restriction on its sales [10]. In adherence to standard prescription, acetaminophen is safe. However, overdose and, or prolonged use of acetaminophen usually result to hepatic and renal damage [11]. Acetaminophen toxicity is primarily attributed to the glutathione depletion by N-acetylp-benzoquinone imine intermediate produced from its breakdown by cytochrome P-450 enzyme which when in excess binds to glutathione or proteins to form glutathione conjugates or acetaminophen-adducts respectively [12, 13]. This will subsequently lead to oxidative stress, triggering generation of superoxide and hydrogen peroxide that will damage to cells, tissues and other biomolecules [14].

Acanthus montanus (Nees) T. Anderson from *Acanthaceae* family is potent medicinal plant called "agamsoso, elele nyiju, and agamefu" in Igbo Language [15]. Its leaves have been used in the treatment of various diseases and health condition including: menstrual cramps or irregularities, bacterial infections, hormonal disorders, cough, urethra discharge, purgative, boils, hypertension, anaemia, arthritis, asthma and urinary tract infections [9, 16, 17]. Much of its medicinal properties are due to its rich phytochemical contents such as alkaloids, flavonoids, saponins, cardiac glycosides, tannins, terpenoids, and proteins. Having considered the immense medicinal potentials of *A. montanus* leaves, this study was therefore, designed to evaluate the effects of its methanol extract on the oxidative stress markers and haematological indices in acetaminophen induced oxidative stress in rats.

METERIALS AND METHODS

Collection and identification of plant material

Fresh leaves of *Acanthus montanus* (Nees) T. Anderson from *Acanthaceae* were collected from Forestry Research Institute of Nigeria, Eastern Station, Ahia-Eke Ndume, Abia State, Nigeria and identified by Dr I.K. Ndukwe of the Department of Forestry, College Natural Resources and Environmental Management (CNREM), Michael Okpara University of Agriculture, Umudike (MOUAU) with Voucher number FHI23965.

Equipment

Equipment employed in this study were of improved analytical standard and found in the Biochemistry Laboratory Unit, Michael Okpara University of Agriculture, Umudike and Shalom Laboratory, Nsukka – Nigeria.

Chemicals and reagents

In this study, chemicals and reagents of high analytical grade were used and they include methanol solvent from Sigma – USA, glutathione peroxidase, superoxide dismutase and catalase assay kits from Randox – UK. Paracetamol from May and Baker (M & B) Pharmaceutical Company limited, UK and Silymarin from Medindia Drugs and Medications, India)

Preparation and extraction of plant material

The fresh leaves of *Acanthus montanus* were carefully removed from their stems and hand-picked to remove debris, every other unwanted material and washed with clean running water to remove dust particles and possible contaminants that may be deposited on the surface of the leaves. The leaves were dried under shade at room temperature for four weeks after which the dried sample was ground into coarse powder. A quantity, 507.8g of the ground sample was first soaked in 1.6 L of n-hexane for 72 h, filtered first with a mesh cloth and subsequently the resulting filtrate was re-filtered with Whatman No.1 filter paper. The residue was dried again under shade for 7 days, reweighed and re-extracted with 1.5 L of absolute methanol for 72 h. The filtrate was concentrated to semisolid using water bath set at 50°C till all the methanol has evaporated.

Experimental animals

Forty-five (45) male Wistar rats weighing 120 – 140 g were obtained from the Animal House, Department of Zoology and Environmental Sciences, University of Nigeria Nsukka, Nigeria and acclimatized for 2

Weeks. The animals were maintained on the conventional animal feed (Vital finisher feed®) with free access to water *ad libitum* in 12 h light/dark cycle throughout the acclimatization period in a very clean environment.

Chempathology analysis

Upon acclimatization, the rats were randomly distributed into 9 groups (n = 5). Group 1 was normal control rats that received distilled water (2 ml/kg) orally for 14 days. Oxidative stress was induced in animals in groups 2, 3, 6, 7, 8 and 9 using oral administrations of acetaminophen (2500 mg/kg) on days 1 and 7. Group 2 (negative control) was oxidative stress induced untreated rats while group 3 (standard control) was oxidative stress induced rats treated with standard drug (silymarin, 100 mg/kg). Group 4 and 5 rats were not oxidative stress induced but treated with 200 and 500 mg/kg body weight of methanol extract of *A. montanus* leaves respectively. Groups 6 – 7 were rats pre-treated with 200 and 500 mg/kg of methanol extract of *A. montanus* leaves respectively every 24 h for 7 consecutive days. After 30 min of administration of the extract on the 7th day, group 6 and 7 were oxidative stress induced using oral administration of acetaminophen (2500 mg/kg body weight) and received 200 and 500 mg/kg body weight of the methanol extract from day 8 – 14. Groups and 9 were oxidative stress induced rats treated with 200 and 500 mg/kg body weight of methanol extract of *A. montanus* leaves respectively every 24 h. Treatments for group 3, 8 and 9 on day 1 and 7 were given 30 min after acetaminophen administration. The study lasted for 14 days and on the 15th day, blood samples were collected for haematological and biochemical analyses.

Biochemical and haematological analyses

Glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) activities were assayed as outlined in the methods of Ursini *et al*., Xin *et al*., and Aebi respectively [18, 19, 20]. Malondialdehyde concentration was determined according to the method described by Wallin *et al*., [21]. Haematological Parameters (haemoglobin concentration, packed cell volume, white blood cell count and red blood cell count were determined according to the methods described by Dacie and Lewis [22].

Statistical analysis

The data obtained were statistically analysed using one-way analysis of variance (ANOVA) with the aid of Statistical Product and Service Solutions (SPSS) version 21. The results were presented as mean ± standard deviation (n = 5). Duncan's multiple comparison post Hoc test (LSD) was used to compare and separate the various means with the acceptable level of significance at $P < 0.05$.

RESULTS

The percentage yield obtained from the extraction of 507.8 g of coarsely ground sample of *Acanthus montanus* leaves with 1.5 L of absolute methanol was 5.42 % equivalent to 27.1 g. This showed that the plant extract is rich in polar constituents as much of them were extractable with methanol solvent. Our previous studies on the methanol extract of *A. montanus* leaves showed that the extract was safe for consumption and no additional acute toxicity of the extract was repeated in this study [23].

The glutathione peroxidase (GPx) activities in Figure 1 showed that there were significant ($P < 0.05$) decrease in the GPx activities of group 2 (negative control), group 3 (positive control) and group 4 – 9 rats when compared with the normal control rats. The normal control, groups 3, 4, 5, 7, 8 and 9 showed significant ($P < 0.05$) increase in GPx activities when compared with negative control while group 6 showed no significant ($P > 0.05$) increase in GPx activity relative to the negative control. More so, normal control, group 4, 5, 8 and 9 showed significant ($P < 0.05$) increase in GPx activities when compared with the positive control while the negative control and group 7 showed significant ($P < 0.05$) and no significant ($P > 0.05$) decrease in GPx activities relative to the positive control respectively.

In the Figure 2, group 2 (negative control), group 3 (positive control), groups 4, 5, 7 and 8 showed significant ($P < 0.05$) decrease in superoxide dismutase (SOD) activities when compared with the normal control whereas groups 9 showed no significant ($P > 0.05$) decrease in SOD activities relative to the normal control. There were significant ($P < 0.05$) increase in SOD activities of normal control, positive control and groups 4 – 9 rats when compared with the negative control. Furthermore, the normal control, groups 4, 8, and 9 showed significant ($P < 0.05$) increase in SOD activities when compared with the positive control. Group 5 and 6 showed no significant ($P > 0.05$) increase in SOD activities with respect to the positive control

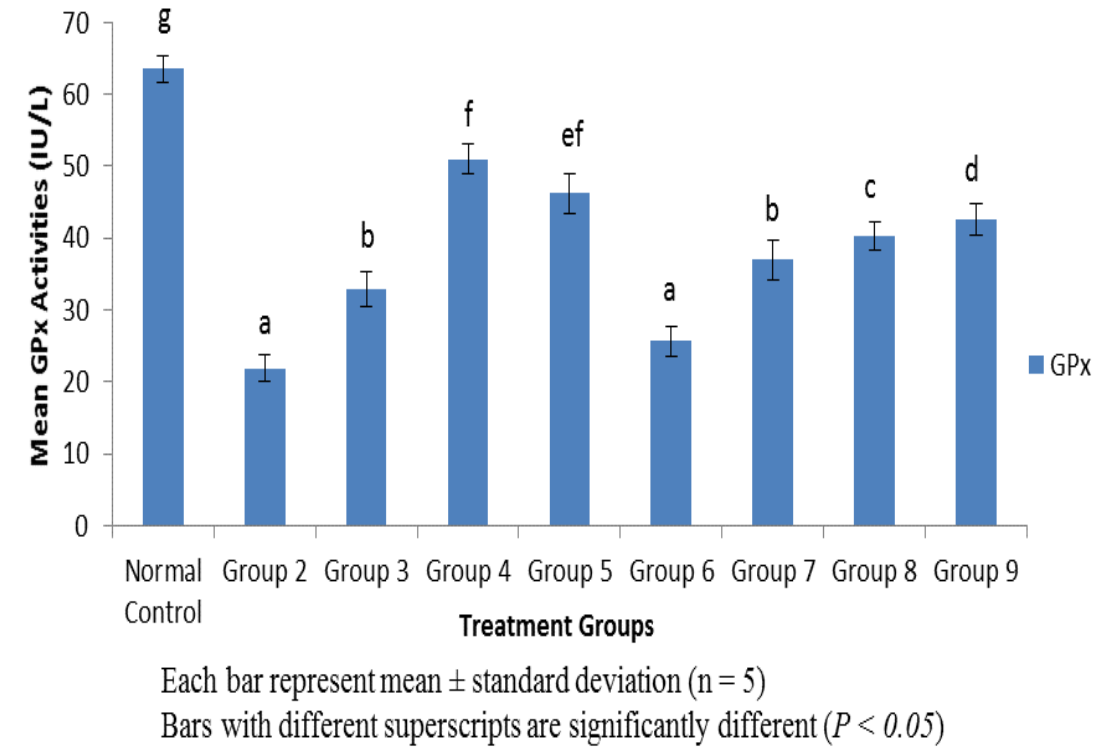

Figure 1: Glutathione peroxidase (GPx) activities in oxidative-stress induced rats treated with methanol extract of *Acanthus montanus* leaves.

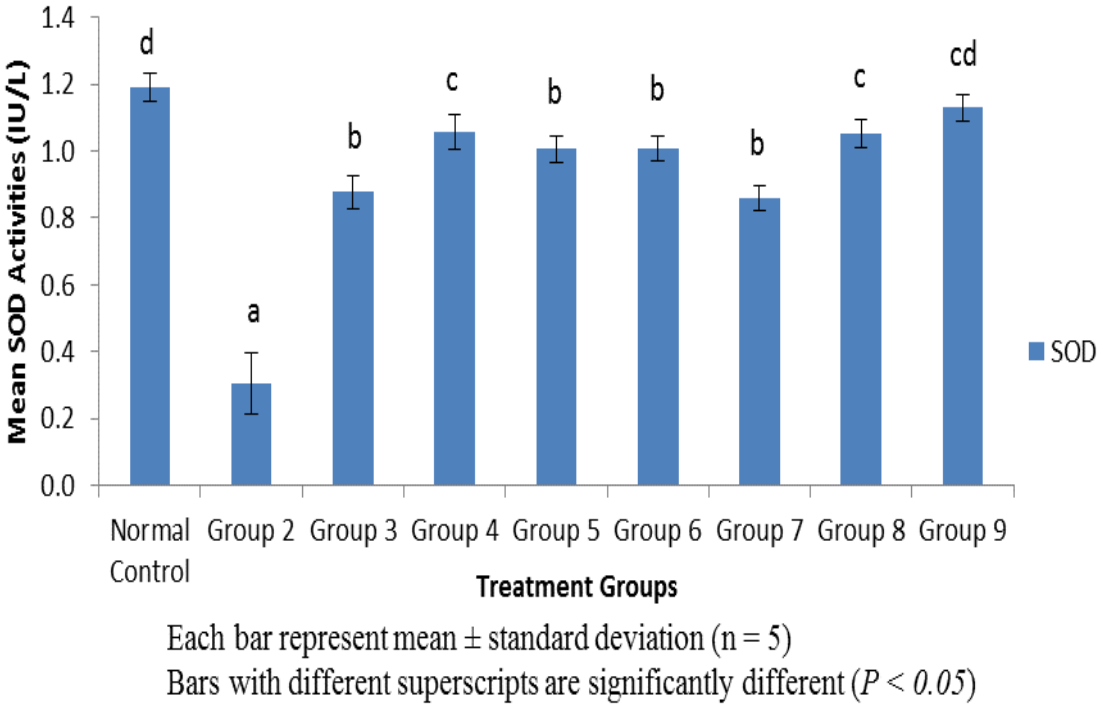

Figure 2: Superoxide dismutase (SOD) activities of oxidative stress induced rats treated with methanol extract of *Acanthus montanus* leaves.

while the SOD activity of group 7 was no significantly ($P > 0.05$) lower than the positive control treated with silymarin.

It was observed that the rats in groups 2 – 9 showed significant ($P < 0.05$) decrease in catalase activities when compared with the normal control (Figure 3). Also, significantly ($P < 0.05$) high catalase activities were observed in the normal control and group 3 – 9 rats when compared with the negative control. In addition, normal control, group 4, 5 and 9 rats showed significant ($P < 0.05$) increase in catalase activities when compared with the positive control. The negative control and group 6 showed significant ($P < 0.05$) decrease in catalase activities relative to the normal control while group 7 and 8 showed no significant ($P > 0.05$) increase and decrease in catalase activities when compared with the positive control respectively.

Group 2 (negative control), group 3 (positive control), groups 6, 7 and 9 showed significant ($P < 0.05$) increase in malondialdehyde (MDA) concentrations when compared with the normal control (Figure 4). However, groups 4, 5, and 8 showed no significant ($P > 0.05$) increase in MDA concentrations when compared with the normal control. The MDA concentrations observed in the normal control, positive control, groups 4, 5, 8, and 9 were significantly ($P < 0.05$) low when compared with the negative control. On the contrary, group 6 and 7 showed no significant ($P > 0.05$) decrease in MDA concentrations when compared with the negative control. The MDA concentrations observed in the normal control was significantly ($P < 0.05$) low when compared with positive control while the negative control had significantly ($P < 0.05$) higher MDA concentration relative to the positive control. Also, groups 6 and 7 showed no significant ($P > 0.05$) increase in MDA concentrations when compared with positive control whereas groups 4, 5, 8 and 9 showed no significant ($P > 0.05$) decrease in MDA concentrations relative to the positive control.

The red blood cell (RBC) counts showed that group 4 and 5 rats that were not oxidative stress induced but received methanol extract of *A. montanus* leaves, 200 and 500 mg/kg body weight respectively showed no significant ($P < 0.05$) decrease in the RBC count when compared with the normal control (Figure 5). However, groups 6 – 7 (protective groups) and groups 8 – 9 (curative groups) showed significant ($P < 0.05$) decrease in RBC counts relative to the normal control. Groups 4, 5, 7, 8 and 9 showed significant ($P < 0.05$) increase in RBC counts when compared with the negative control while the RBC count of group 6 was no significantly ($P < 0.05$) higher than the RBC count of the negative control (group 2). The RBC counts of the normal control, groups 4, 5, 8 and 9 were significantly ($P < 0.05$) higher than the RBC count of the positive control (group 3) treated with silymarin while that of the negative control (group 2) was significantly ($P < 0.05$) lower than the positive control. It was also observed that the RBC counts of group 6 and 7 showed no significant ($P > 0.05$) decrease and increase respectively when compared with the RBC count of the positive control.

The data in Figure 6 showed that there were significant ($P < 0.05$) decrease in the white blood cell (WBC) counts in the negative control (group 2) that was paracetamol induced but untreated and positive control (group 3) that was paracetamol induced but treated with silymarin, when compared with normal control. Group 4 and 5 rats that were not paracetamol induced but received graded doses of methanol extract of *A. montanus* leaves, group 6-7 (protective groups) and group 8-9 (curative groups) that were paracetamol induced but treated with graded doses of methanol extract of *A. montanus* leaves showed significant ($P < 0.05$) decrease in WBC counts when compared with normal control. There were significant ($P < 0.05$) increase in the WBC counts of the normal control and group 3 – 9 when compared with the WBC of the negative control. The WBC counts of the normal control, and group 4 showed significant ($P < 0.05$) increase when compared with positive control while the negative control showed significant ($P < 0.05$) decrease in WBC count relative to the positive control. Group 6 and 7 showed no significant ($P > 0.05$) decrease in WBC count when compared with the positive control. There was no significant ($P > 0.05$) difference between the WBC counts group 8 and positive control while group 9 showed no significant ($P > 0.05$) increase in WBC count when compared with the positive control.

Group 2 (negative control), group 3 (positive control), group 6, 7 and 8 showed significant ($P < 0.05$) decrease in the percentage packed cell volume (PCV) when compared with the normal control while group 4, 5, and 9 showed no significant ($P > 0.05$) decrease in PCV relative to the normal control (Figure 7). The normal control and group 3 – 9 showed significant ($P < 0.05$) increase in PCV when compared with negative control (group 2). In addition, normal, group 4, 5, and 9 showed significant ($P < 0.05$) increase in PCV when compared with the positive control while group 2 showed significant ($P < 0.05$) decrease in

PCV relative to the positive control. Group 6, 7 and 8 showed no significant ($P > 0.05$) decrease in PCV when compared with the positive control.

There were significant ($P < 0.05$) decrease in the haemoglobin (Hb) concentrations of the negative control when compared with the normal control (Figure 8). Whereas, group 3, 6, 7 and 8 showed no significant ($P > 0.05$) decrease in Hb concentrations when compared with the normal control while group 4, 5, and 9 showed no significant ($P > 0.05$) increase in Hb concentrations relative to the normal control. It was also observed that the normal control, group 4, 5 and 9 showed significant ($P < 0.05$) increase in Hb concentrations when compared with the negative control (group 2) while group 3 (positive control), 6, 7 and 8 showed no significant ($P > 0.05$) increase in Hb concentrations relative to the negative control. More so, normal control, group 4, 5, 7 and 9 showed no significant ($P > 0.05$) increase in Hb concentrations when compared with positive control while group 2, 6, and 8 showed no significant ($P > 0.05$) decrease in Hb concentrations when compared with the positive control.

DISCUSSION

In this study, effects of methanol extract of *Acanthus montanus* leaves on oxidative stress markers and haematological parameters in rats; induced oxidative stress induced with high doses of acetaminophen were investigated. This was to understand its ameliorative potentials in the management of oxidative stress and maintenance of healthy haematological functions. Acetaminophen commonly known as paracetamol is an analgesic and antipyretic drug that has been established to be toxic to various tissues and organs in the body including liver and kidney when taken in excess dose due to oxidative activity of its metabolites, N-acetyl-p-benzoquinone imine [23]. N-acetyl-p-benzoquinone imine in excess amount interacts with proteins and antioxidant enzymes with sulfhydryl group such as glutathione peroxidase and causes its depletion. Thus, depletion of glutathione and its derivatives makes biomolecules, tissues and organs more vulnerable to N-acetyl-p-benzoquinone imine attack and increased lipid peroxidation and inevitably resulting in oxidative stress and its associated health consequences [24].

The significant ($P < 0.05$) reduction in the antioxidant enzymes (glutathione peroxidase, superoxide dismutase and catalase) activities and significant ($P < 0.05$) increase in lipid peroxidation (malondialdehyde concentration) in the oxidative stress induced untreated rats are due to the effects of N-acetyl-p-benzoquinone imine radical attack. The excess of the N-acetyl-p-benzoquinone imine generated from metabolism of overdose of acetaminophen could have bound to glutathione which is a major cofactor of glutathione peroxidase (GPx) and depleted it. In addition, excess N-acetyl-p-benzoquinone imine from previous studies have been shown to bind to proteins and form N-acetyl-p-benzoquinone imine – protein adduct (acetaminophen-adduct) which deplete the amount of active circulating antioxidant enzymes further exposing the body to free radical attack and lipid peroxidation. Thus, the oxidative stress caused destruction; reduction and malfunctioning of the antioxidant enzymes in untreated rats making the rats suffer increased level of lipid peroxidation as demonstrated by the high levels of malondialdehyde concentrations in the rats. The reduction in antioxidant enzyme activities and increase in malondialdehyde concentrations observed in non-oxidative stress induced rats (group 4 and 5) showed that the methanol extract of *A. montanus leaves* could result to decrease in antioxidant enzymes when taken under non oxidative stress condition. These observed effects of the methanol on the antioxidant enzymes are similar to effects of many well-known drugs when taken without due prescription for therapeutic purposes, and thus it should be consumed only for therapeutic purpose to prevent its abuse and any possible health effects. The increased level of malondialdehyde concentration in the acetaminophen induced rats is attributed to oxidative stress elicited by the excess N-acetyl-p-benzoquinone imine produced from acetaminophen breakdown due to inability of antioxidants in body to quench its action as has been shown by various studies [24]. These reductions in antioxidant enzymes activities are in agreement with the findings of Didunyemi *et al*., that acetaminophen induced oxidative stress results in drastic reductions in catalase, superoxide dismutase and glutathione peroxidase activities relative to normal control [25].

However, the significant ($P < 0.05$) improvement observed in the antioxidant enzymes (glutathione peroxidase, superoxide dismutase and catalase) activities and reduction in the level lipid peroxidation product (malondialdehyde) in the oxidative stress induced rats treated with various doses of the *A. montanus leaves* extract could be attributed to the antioxidant properties of the phytoconstituents of

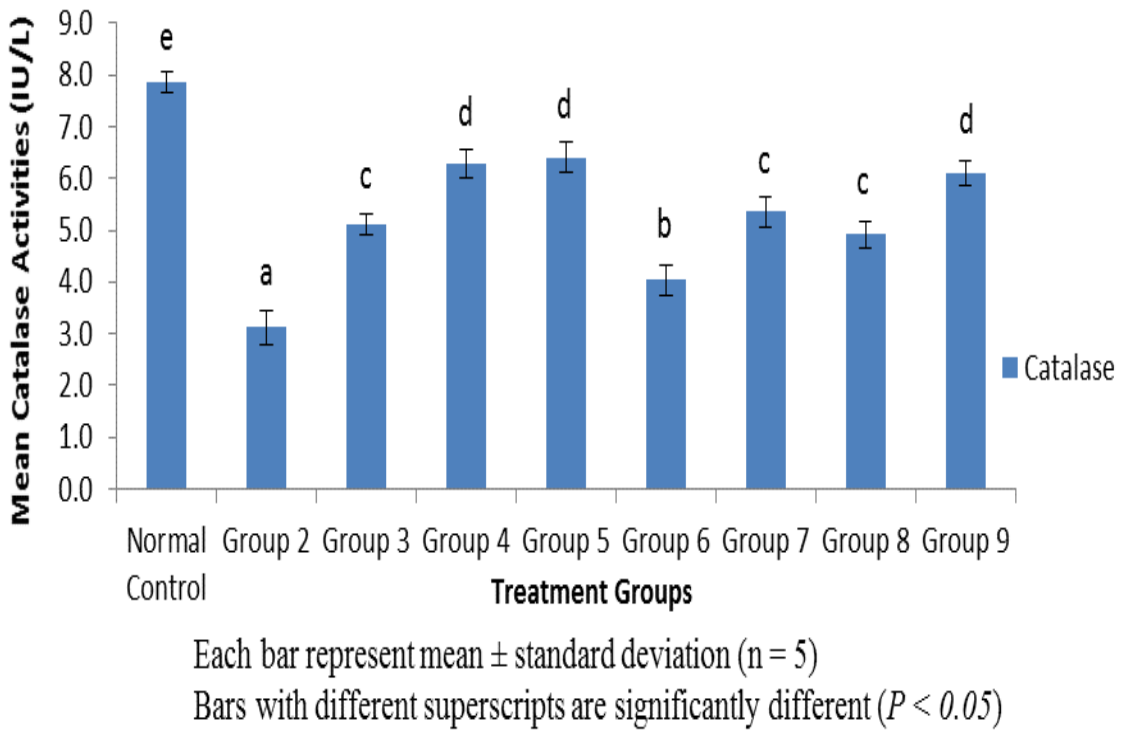

Figure 3: Catalase activities of oxidative stress induced rats treated with methanol extract of *Acanthus montanus* leaves.

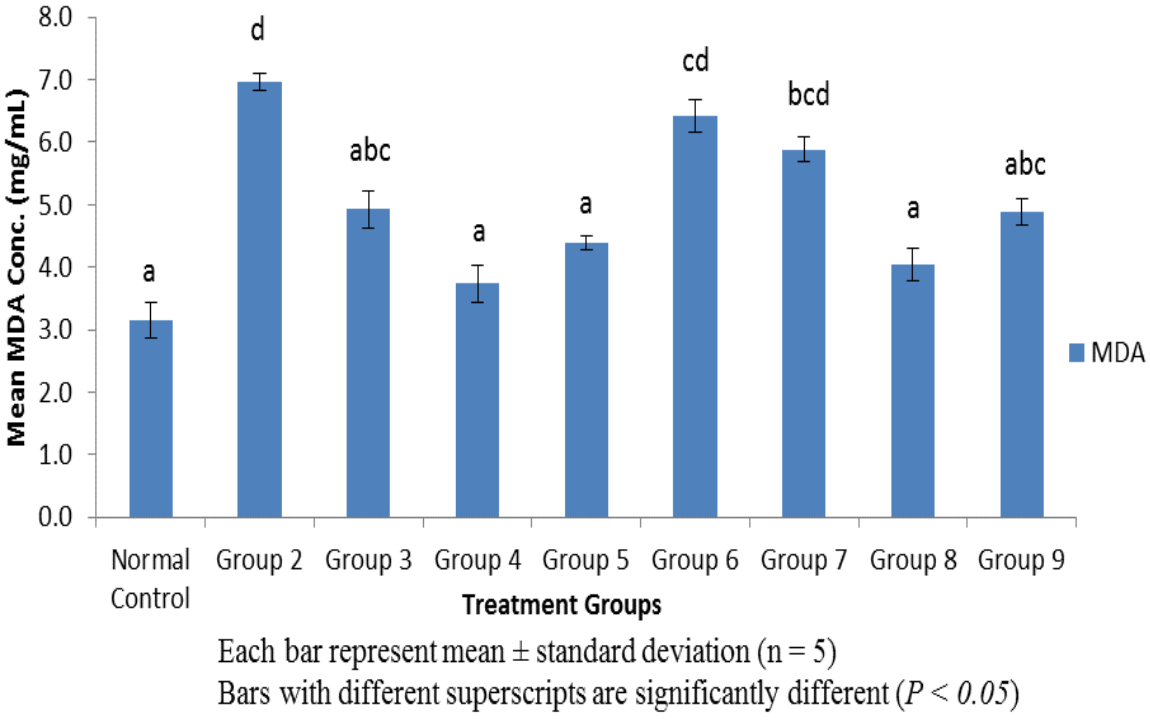

Figure 4: Malondialdehyde concentrations (MDA) in oxidative stress induced rats treated with methanol extract of *A. montanus* leaves.

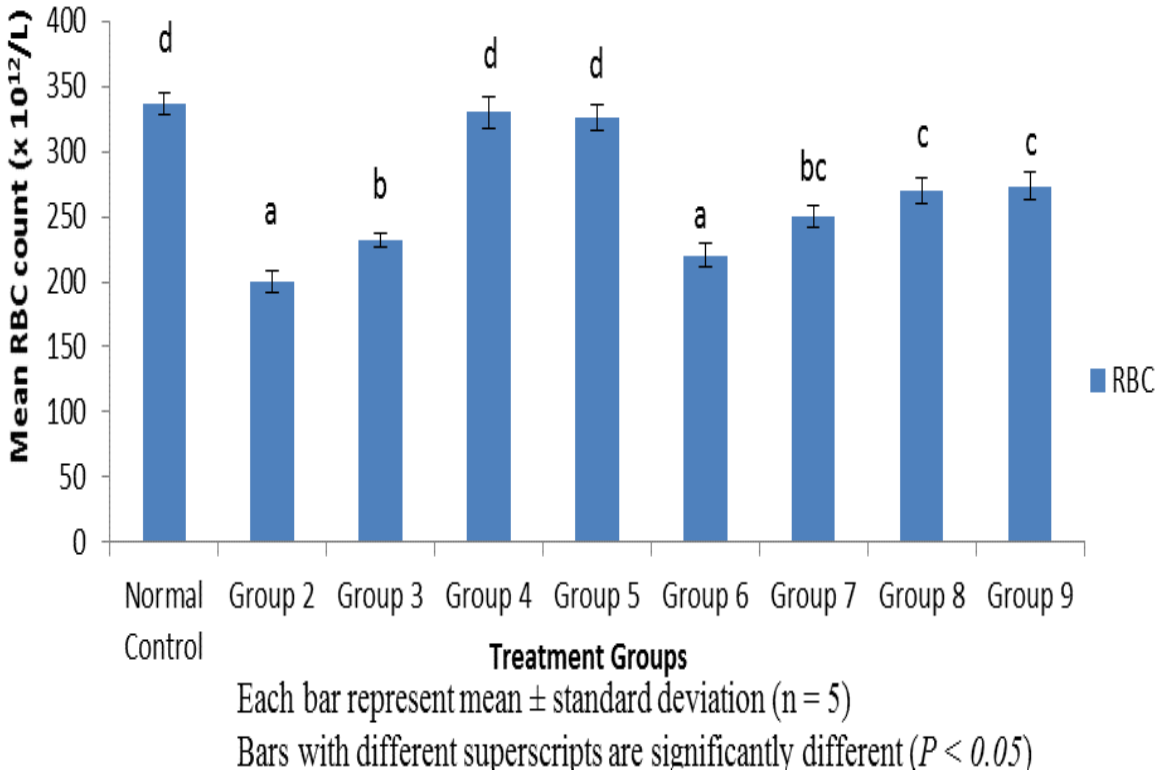

Figure 5: Red blood cell (RBC) count of oxidative stress induced rats treated with methanol extract of *A. montanus* leaves.

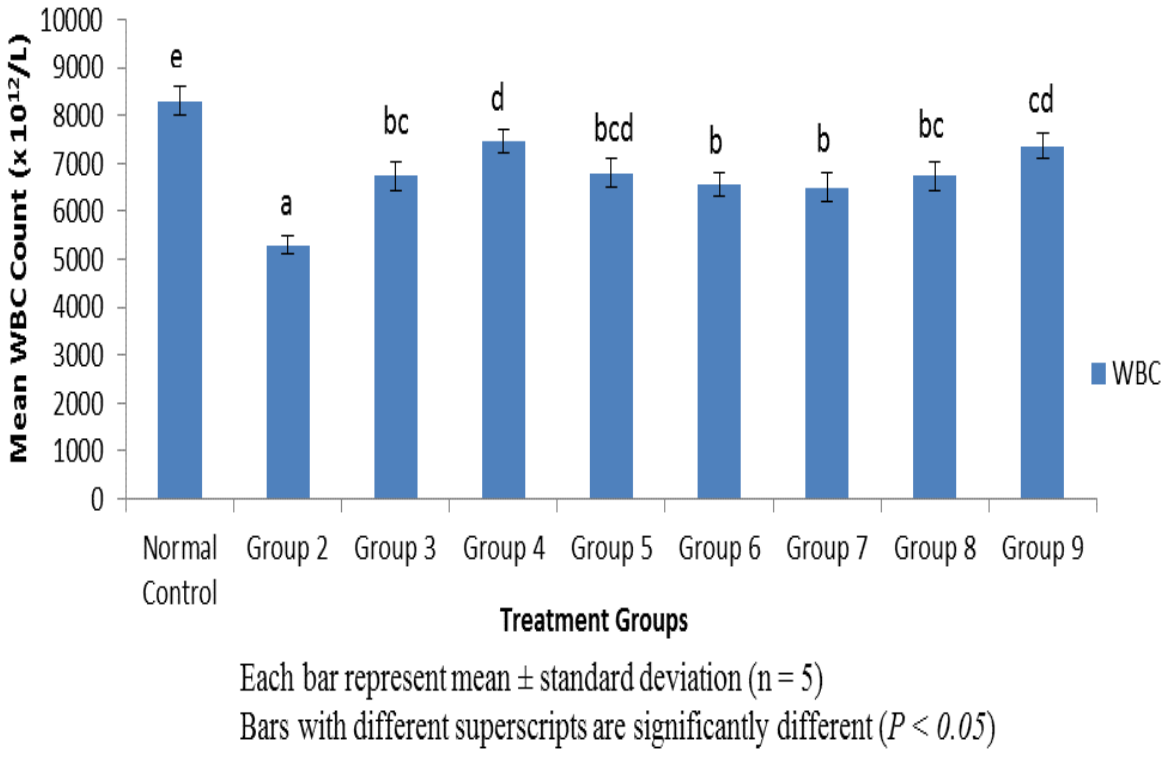

Figure 6: White blood cell (WBC) count of oxidative stress induced rats treated with methanol extract of *A. montanus* leaves.

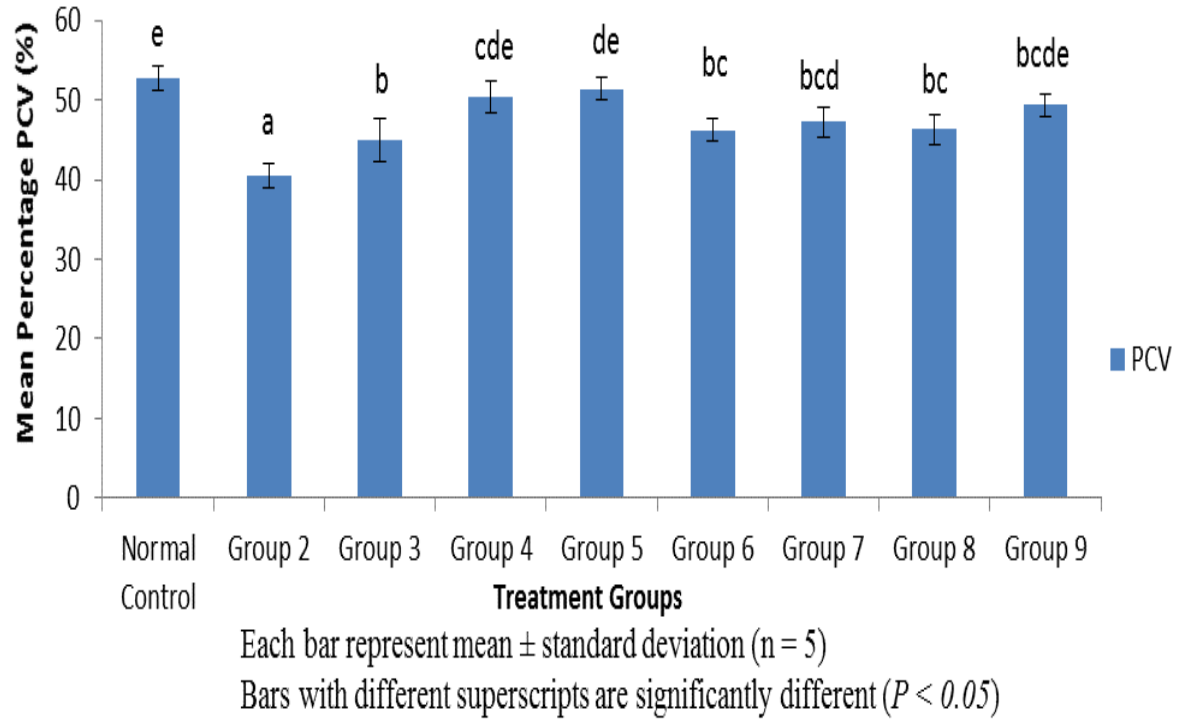

Figure 7: Packed cell volume (PCV) of oxidative stress induced rats treated with methanol extract of *A. montanus* leaves.

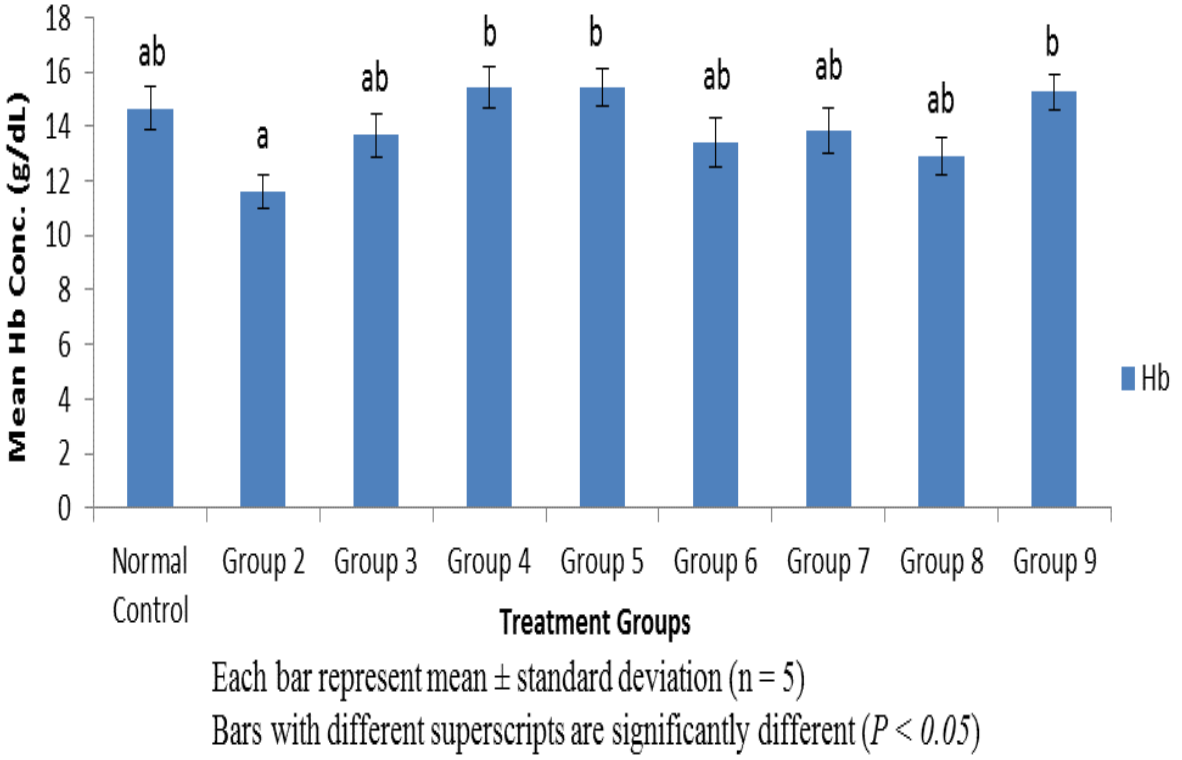

Figure 8: Haemoglobin (Hb) concentrations of oxidative stress induced rats treated with methanol extract of *A. montanus* leaves.

the extract. This is in agreement with the findings from other studies that antioxidants are able to reverse oxidative stress via their free radical scavenging activities, prevention of lipid peroxidation and stimulation of increased synthesis of antioxidant enzymes [26]. The bioactive antioxidants present in the extract were able to attenuate N-acetyl-p-benzoquinone imine attack on biomolecules, tissues, organs and prevented lipid peroxidation. This could have made possible by non-enzymatic antioxidants such as vitamins C and E, phenols, carotenoids and flavonoids that are responsible for most antioxidant activities exhibited by medicinal plant extracts. Plant extracts rich in antioxidant compounds can induce increase expression of genes coding for antioxidant enzymes and their stability, inhibit generation of free radicals and prevent initiation and propagation of free radical attack on biomolecules. The *A. montanus leaves* extract could have in addition to these mechanisms, prevented formation Acetaminophen-protein adduct that would have depleted antioxidant enzymes and increased N-acetyl-p-benzoquinone imine attack, thus sparing biomolecules and tissues from oxidative stress and lipid peroxidation. The reversal of the decline in the catalase, superoxide dismutase and glutathione peroxidase activities and reduction in the level of lipid peroxidation observed in the methanol extract of *A. montanus* leaves treated groups are indication of recovery of the rats from oxidative stress and associated adverse health effects in line with the findings of Didunyemi *et al*., [26]. Thus, compounds or plant extracts that reduce oxidative stress attack on living organism as demonstrated by the methanol extract of *A. montanus leaves* in this study can promote normal health, reduce complications arising from oxidative stress due to their effectiveness, low cost, less side effects and ease of availability.

Haematological indices are adversely affected in individuals experiencing oxidative stress that in most cases such individuals suffer anaemia, red blood cells abnormalities, decrease in packed cell volume and haemoglobin concentration, tissues and organ failure. The reductions in the haematological parameters (red blood cell count, packed cell volume, haemoglobins and white blood cell counts) in the oxidative stress induced rats but untreated are indications of N-acetyl-p-aminophenol imine attack on the rats. The N-acetyl-p-aminophenol imine could have caused damage on the red blood cells and white blood cells which resulted to their rapid destruction and decrease synthesis by the erythropoietic cells. The reductions in the red blood cells and packed cell volume led to decreased haemoglobin concentrations and resultant anaemia in the oxidative stress induced untreated rats. These cause decrease in the ability of the rats to transport oxygen efficiently. The reduction in the white blood cells in the oxidative stress induced rats was contrary to some reports that acetaminophen induced oxidative stress caused increase in white blood cells as inflammatory response to the stress which is an agreement with the earlier findings by Oyedeji *et al*., [27]. Treatments of the oxidative stress induced rats with graded doses of methanol extract of *A. montanus* leaves greatly improved showing that the rats were recovering from haematoxic effects of N-acetyl-p-aminophenol imine. The increased levels of percentage PCV, WBC counts, RBC counts and Hb concentrations in the extract treated groups will promote normal haematological functions and help the rats to survive the toxic effects of N-acetyl-p-aminophenol imine attack generated acetaminophen breakdown to great extent. The bioactive phytoconstituents of the extract could have stabilized, the circulating red blood cells, white blood cells, or stimulate erythropoiesis leading to increased synthesis of red blood cells and packed cell volume which will inevitably translate into increased level of Hb concentrations as observed.

CONCLUSIONS

The findings of this study show that methanol extract of *Acanthus montanus* leaves possesses antioxidative and blood replenishing properties that were able to ameliorate oxidative stress and anaemia induced by excess acetaminophen administration. However, further research is required to identify and isolate the bioactive components responsible for these activities in order to maximize its therapeutic potentials.

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