

## Full Length Research Paper

# Ethanol extracts of *Newbouldia laevis* stem and leaves modulate serum liver marker enzymes and antioxidant enzymes activities in diabetic rats

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Diabetes is known to involve oxidative stress and changes in antioxidant enzymes. Many plants metabolites have been shown to possess antioxidant activities, improving the effects of oxidative stress complications in diabetic conditions. This study evaluated the effects of extracts from *Newbouldia laevis* leaves and stem on liver marker enzymes and antioxidant enzymes in rat model. The results confirmed that the untreated diabetic rats were subjected to oxidative stress as indicated by significantly abnormal activities of their scavenging enzymes (low superoxide dismutase, catalase and glutathione activities) to the extent of liver enzymes leakage from the hepatocytes when compared with apparently healthy rats. The ethanol extracts of *N. laevis* leaves and stem possessed antioxidant activity as shown by increased activities of superoxide dismutase and catalase, and glutathione levels of the diabetic rats after treatment. High levels of alkaline phosphatase (ALP), and alanine aminotransaminase (ALT), which are typical of oxidative stress condition were differentially ameliorated after treatment with the ethanol extracts of *N. laevis* leaves and stem in a dose dependent manner.

**Key words:** Diabetes mellitus, oxidative stress, *Newbouldia laevis*, liver enzymes, antioxidant enzymes.

## INTRODUCTION

Oxidative stress is a term used to refer to the shift towards the pro-oxidants in the pro-oxidants/antioxidants balance that can occur as a result of an increase in oxidative metabolism (Manda et al., 2009). Reactive oxygen species (ROS) reactions with biomolecules such as lipid, protein and DNA, produce different types of secondary radicals like lipid radicals, sugar and base derived radicals, amino acid radicals depending on the

nature of the ROS (Niki et al., 2005). These radicals in the presence of oxygen are converted to peroxy radicals. Peroxy radicals are critical in biological systems, as they often induce chain reactions. These reactions exert oxidative stress on the cells, tissues and organs of the body. The biological implications of such reactions depend on several factors like site of generation, nature of the substrate, activation of repair mechanisms, and

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redox status among many others (Koppeno, 1993; Goldstein et al., 1993). Oxidative stress is increased in diabetes mellitus owing to an increase in the production of oxygen free radicals and insufficiency in antioxidant defense mechanisms (Lawrence et al., 2008; Soliman, 2008). Alloxan is a toxic glucose analogue, which selectively destroys insulin-producing cells in the pancreas (that is beta cells) when administered to rodents and many other animal species (Szkudelski, 2001). This causes an insulin-dependent diabetes mellitus (called "Alloxan Diabetes") in these animals, with characteristics similar to type 1 diabetes in humans. Alloxan is selectively toxic to insulin-producing pancreatic beta cells because it preferentially accumulates in beta cells through uptake via the GLUT2 (glucose transporter 2) (Szkudelski et al., 1998). Alloxan, in the presence of intracellular thiols, generates reactive oxygen species (ROS) in a cyclic reaction with its reduction product, dialuric acid (Stanley and Venugopal, 2001). The beta cell toxic action of alloxan is initiated by free radicals formed in this redox reaction (Szkudelski, 2001).

Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function (Mittler, 2002). Fortunately, free radical formation is controlled naturally by various beneficial compounds known as antioxidants. It is when the availability of antioxidants is limited that this damage can become cumulative and debilitating (Cheeseman and Slater, 1993; Sies, 1997). Antioxidants are our first line of defense against oxidative damage, and are critical for maintaining optimum health and well-being. Oxidative stress is increased in diabetes mellitus owing to an increase in the production of oxygen free radicals, such as super oxide ( $O_2^{\cdot-}$ ), hydrogen peroxide ( $H_2O_2$ ) and hydroxide ( $OH^{\cdot}$ ) radicals which overwhelm the natural antioxidant defence mechanisms (Soliman, 2008). Antioxidants are capable of stabilizing, or deactivating, free radicals before they attack cells. Antioxidants are absolutely critical for maintaining optimal cellular and systemic health and well-being (Traber and Atkinson, 2007). To protect the cells, organ and systems of the body against reactive oxygen species, humans have evolved a highly sophisticated and complex antioxidant protection system (Vertuani et al., 2004).

*Newbouldia laevis* is widely used in African folk medicine for the treatment of malaria and fever, stomach-ache, coughs, sexually transmitted diseases, tooth ache, breast cancer, and constipation (Iwu, 1983). In south eastern and part of the Midwestern Nigeria, the plant is used for the treatment of septic wounds and eye problems (Akerlele et al., 2011). In Nigeria, the bark is chewed and swallowed for stomach pains, diarrhea and toothache (Iwu, 2000). It has also been found useful for children's convulsion (Akunyili, 2000). The antimicrobial potential of methanol extract of the leaf (Kutete et al., 2007; Usman and Osuji, 2007; Ejele et al., 2012) have been reported in literature while the anti-inflammatory

(Usman et al., 2008) and anti-malarial (Gbeassor et al., 2006) activities of the root extract have been documented. Sedative effects of the methanol leaf extract of *N. laevis* in mice and rats have also been studied and reported (Amos et al., 2002). *N. laevis* is one of such medicinal plants whose medicinal values have stood the test of time. Base on these data, the present study aims to trace how ethanol extracts of *N. laevis* stem and leaves modulate serum liver marker enzymes and antioxidant enzymes activities in alloxan induced oxidative stressed rats.

## MATERIALS AND METHODS

### Collection and identification of plant materials

The leaves and stem of *N. laevis* were used for this study. They were collected within University of Nigeria, Nsukka and were identified by Mr. Alfred Ozioko of Bioresources Development and Conservation Programme (BDCP) Research Centre, Nsukka, Enugu State. The fresh leaves and stem of *N. laevis* were first washed with distilled water and subsequently, normal saline to remove dirt and possible mycotoxins. The samples were dried under shade for several days and then pulverized into fine powder.

### Extraction of plant materials

A quantity, 500 g of each ground sample was macerated in 1.5 L of ethanol for 48 h. The solution was filtered with Whatman no. 4 filter paper and the filtrate was concentrated to a semi-solid residue using a rotary evaporator.

### Animals

Forty two (42) adult albino rats of both sexes were used for this study. All the animals used were obtained from the Animal house of the Department of Zoology, University of Nigeria Nsukka. The rats were fed with standard grower's mash rat pellets (Grand Cereals LTD, Enugu) and water.

### Experimental design and analysis

Forty-two (42) Wistar albino rats of both sexes weighing between 102 and 240 g were used for the study. They were acclimatized for fourteen (14) days with free access to feed and water. After acclimatization, they were evenly distributed into seven (7) groups of six rats each. The route of administration was by oral intubation for a period of twenty one (21) days. The groups and doses administered are summarised below:

Group 1: Control (Normal rats)

Group 2: Positive control (Diabetic untreated rats)

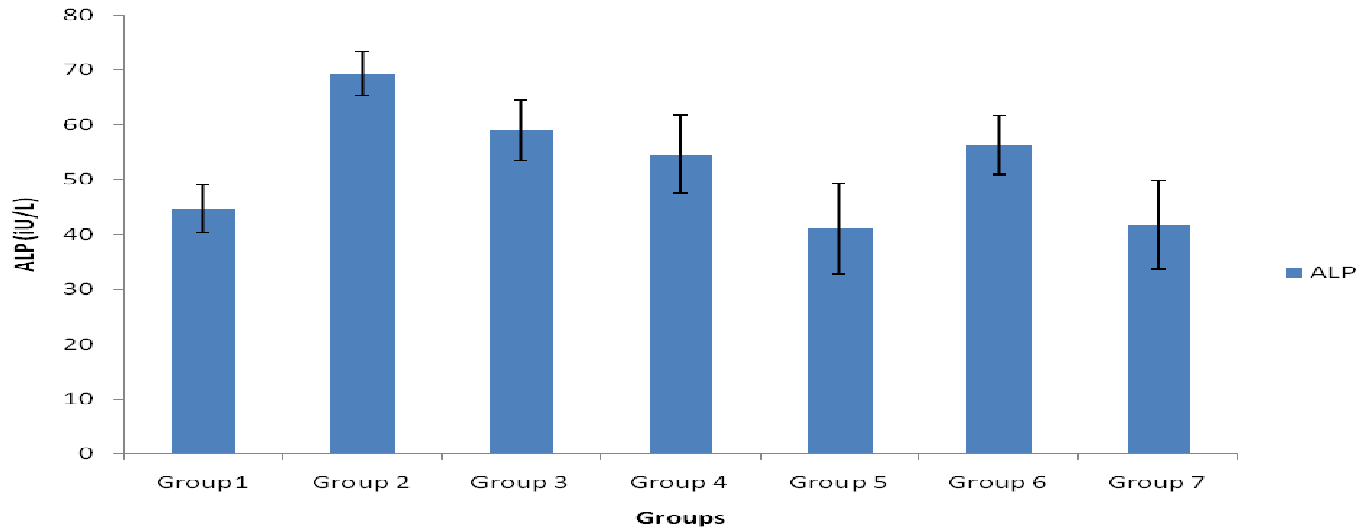
Group 3: Alloxan induced diabetic rats + 2.5 mg/kg body weight of Glibenclamide

Group 4: Alloxan induced diabetic rats + 200 mg/kg body weight of ethanol leaves extract

Group 5: Alloxan induced diabetic rats + 400 mg/kg body weight of ethanol leaves extract

Group 6: Alloxan induced diabetic rats + 200 mg/kg body weight of ethanol stem extract

Group 7: Alloxan induced diabetic rats + 400 mg/kg body weight of ethanol stem extract



**Figure 1.** Effect of ethanol extracts of *Newbouldia laevis* leaves and stems on serum alkaline phosphatase (ALP). Group 1: Control (Normal rats), Group 2: Positive control (Diabetic untreated rats), Group 3: Alloxan induced diabetic rats + 2.5 mg/kg body weight of Glibenclamide, Group 4: Alloxan induced diabetic rats + 200 mg/kg body weight of ethanol leaves extract, Group 5: Alloxan induced diabetic rats + 400 mg/kg body weight of ethanol leaves extract, Group 6: Alloxan induced diabetic rats + 200 mg/kg body weight of ethanol stem extract, and Group 7: Alloxan induced diabetic rats + 400 mg/kg body weight of ethanol stem extract.

At the end of the experimental period the rats were starved for 12 h and then sacrificed under ether anaesthetized. Blood samples were received into clean dry centrifuge tube and left to clot at room temperature, then centrifuged for 10 min at 3000 r.p.m to separate serum. Serum was carefully separated into dry clean Wassermann tubes, using a Pasteur pipette and kept frozen at (-20°C) until estimation of some biochemical parameters.

#### Statistical analysis

Data were presented as mean of three replicates  $\pm$  SD. Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS) version 19. One way analysis of variance was adopted for comparison, and the results were subject to post hoc test using least square deviation (LSD). The data were expressed as mean  $\pm$  standard deviation.  $P < 0.05$  was considered significant.

## RESULTS

### Effect of ethanol extracts of *Newbouldia laevis* leaves and stems on serum alkaline phosphatase (ALP).

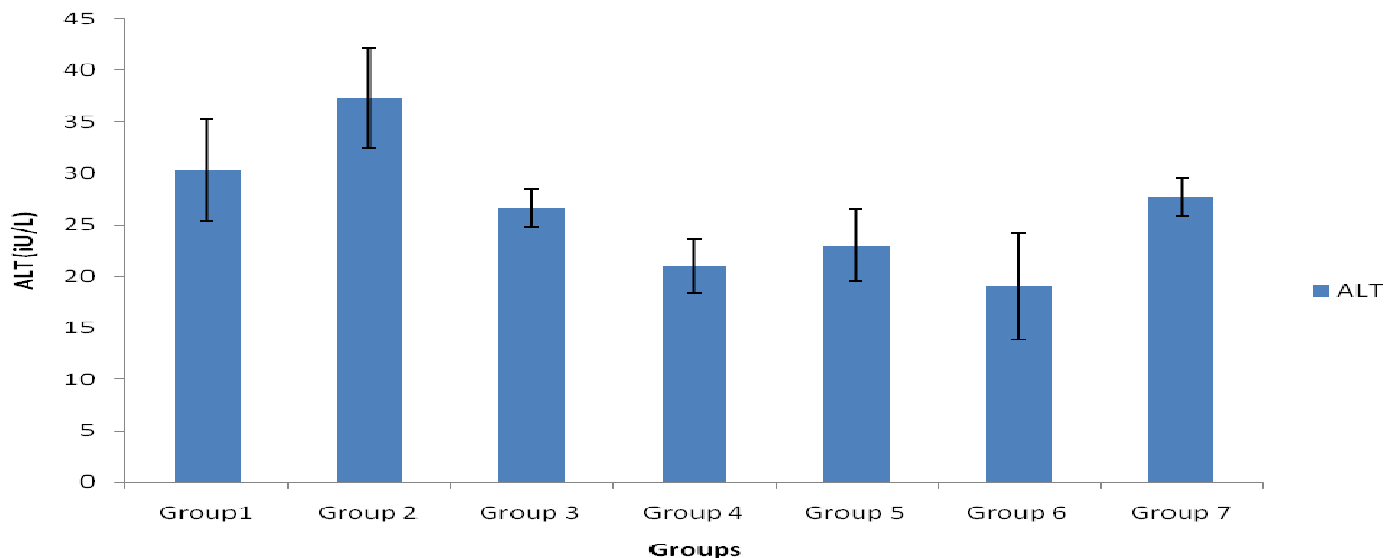
Figure 1 shows that the diabetic untreated group (group 2) had the highest activity of ALP but the administration of the different doses of the extracts resulted in significant ( $p < 0.05$ ) decrease in ALP activity. The 200 mg/kg body weight of the extracts (groups 4 and 6) in a similar manner as the standard drug (glibenclamide) significantly and dose dependently reduced the activity of ALP when compared with the values obtained from the diabetic untreated group (group 2). However, the 400 mg/kg body weight of the extracts caused greater reduction in the activity of ALP.

### Effect of ethanol extracts of *Newbouldia laevis* leaves and stems on serum alanine aminotransaminase (ALT)

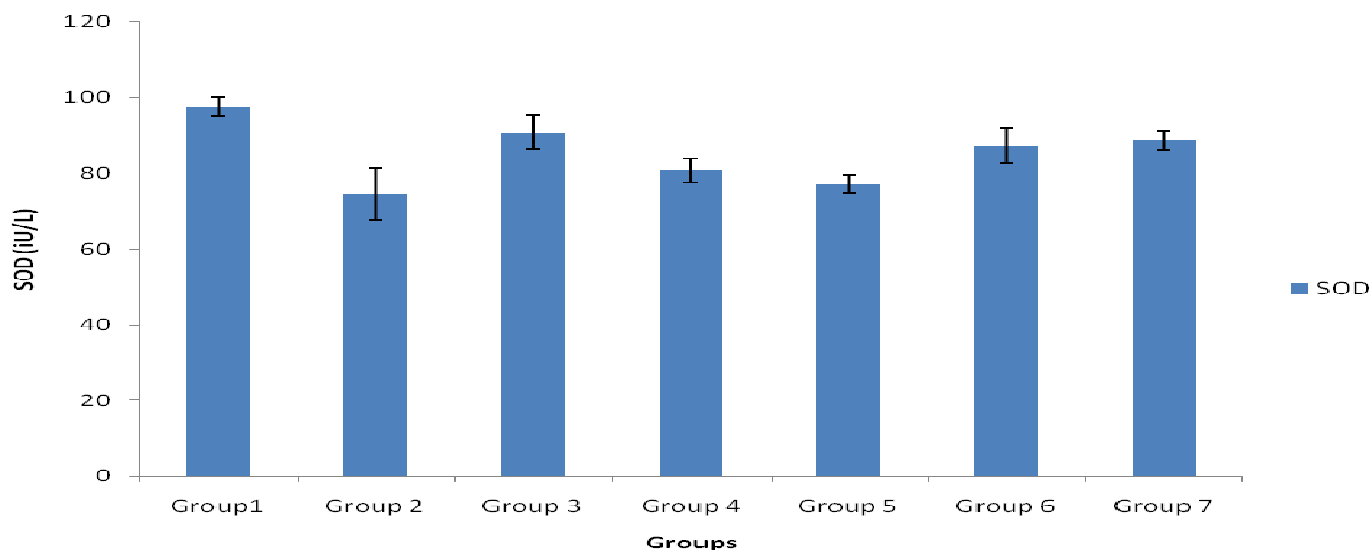
Figure 2 shows that the diabetic untreated group (group 2) had the highest activity of ALT but the administration of the different doses of the extracts resulted in significant ( $p < 0.05$ ) decrease in ALT activity. The 400 mg/kg body weight of the extracts (group 5 and 7) in a similar manner as the standard drug (glibenclamide) significantly and dose dependently reduced the activity of ALT when compared with the values obtained from the diabetic untreated group (group 2). However, the 200 mg/kg body weight of the extracts caused greater reduction in the activity of ALP even more than the standard drug.

### Effect of ethanol extracts of *Newbouldia laevis* leaves and stem on serum superoxide dismutase (SOD) activity

Figure 3 shows that the diabetic untreated group (group 2) had reduced SOD activity but the administration of the different doses of the extracts resulted in significant ( $p < 0.05$ ) increase in SOD activity. The extracts stimulated SOD activity in diabetic rats treated with the leaves and stem. A significant increase ( $p < 0.05$ ) was observed in the SOD activity of rats in the test groups when compared with the positive control. Significant increases ( $p < 0.05$ ) were observed in groups 6 and 7 (stem extract treated) when compared with groups 4 and 5 (leave extract treated).



**Figure 2.** Effect of ethanol extracts of *Newbouldia laevis* leaves and stems on serum alanine aminotransaminase (ALT). Group 1: Control (Normal rats), Group 2: Positive control (Diabetic untreated rats), Group 3: Alloxan induced diabetic rats + 2.5 mg/kg body weight of Glibenclamide, Group 4: Alloxan induced diabetic rats + 200 mg/kg body weight of ethanol leaves extract, Group 5: Alloxan induced diabetic rats + 400 mg/kg body weight of ethanol leaves extract, Group 6: Alloxan induced diabetic rats + 200 mg/kg body weight of ethanol stem extract, and Group 7: Alloxan induced diabetic rats + 400 mg/kg body weight of ethanol stem extract.

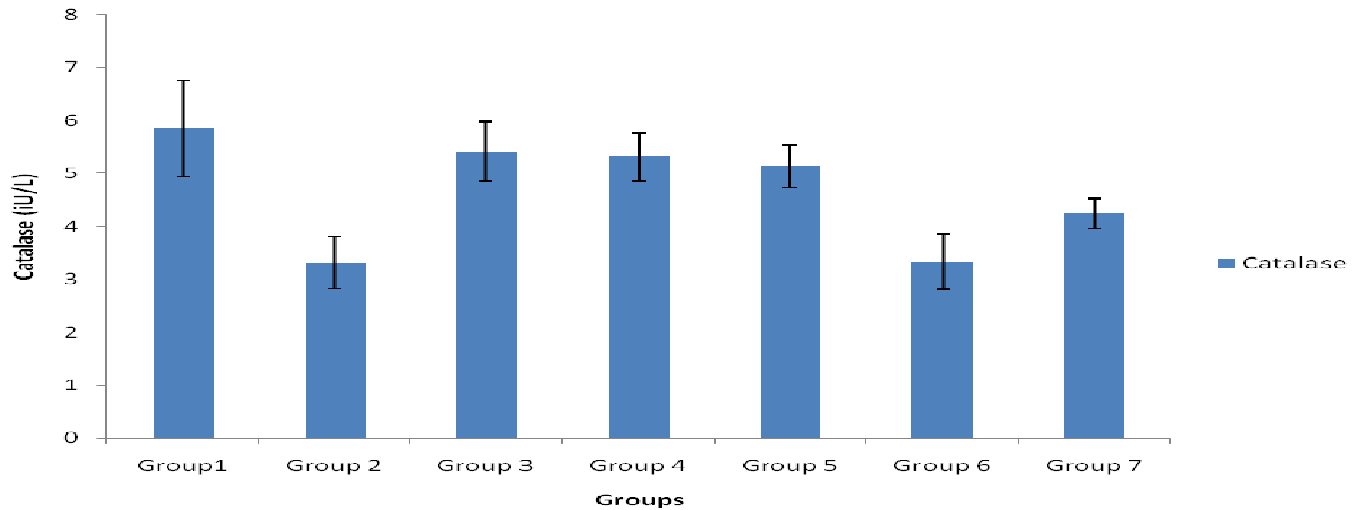


**Figure 3.** Effect of ethanol extracts of *Newbouldia laevis* leaves and stem on serum superoxide dismutase (SOD) activity of diabetic rats. Group 1: Control (Normal rats), Group 2: Positive control (Diabetic untreated rats), Group 3: Alloxan induced diabetic rats + 2.5 mg/kg body weight of Glibenclamide, Group 4: Alloxan induced diabetic rats + 200 mg/kg body weight of ethanol leaves extract, Group 5: Alloxan induced diabetic rats + 400 mg/kg body weight of ethanol leaves extract, Group 6: Alloxan induced diabetic rats + 200 mg/kg body weight of ethanol stem extract, and Group 7: Alloxan induced diabetic rats + 400 mg/kg body weight of ethanol stem extract.

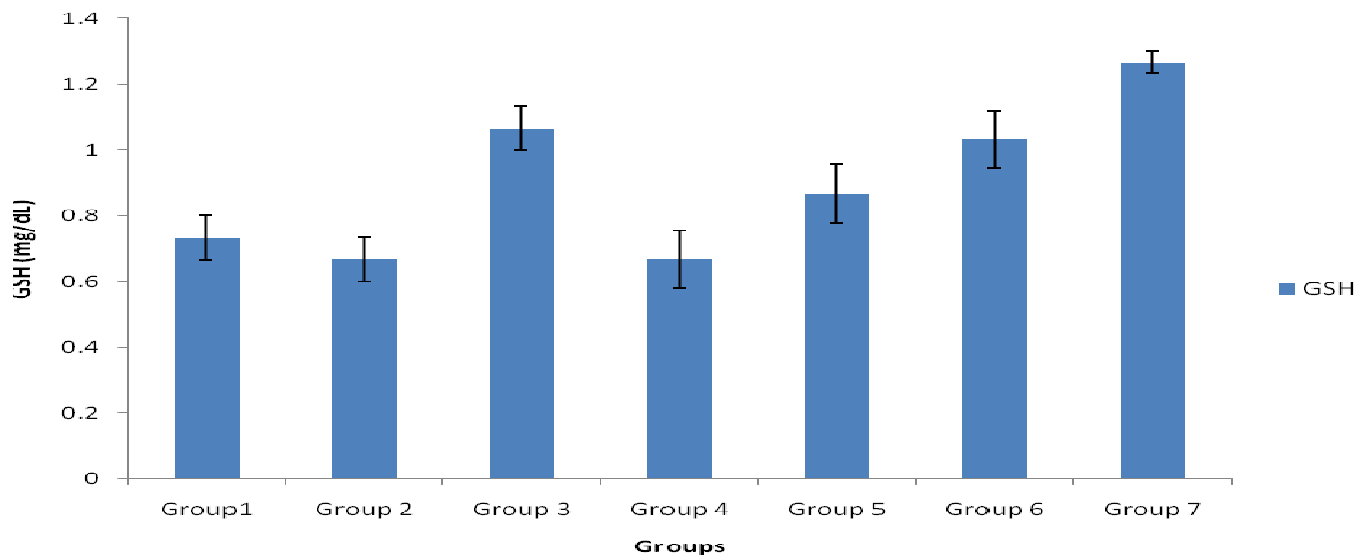
#### Effect of ethanol extracts of *Newbouldia laevis* leaves and stem on serum catalase activity

Figure 4 shows that the diabetic untreated group (group

2) had reduced catalase activity but the administration of the different doses of the extracts resulted in significant ( $p < 0.05$ ) increase in catalase activity of diabetic rats treated with the leaves and stem extracts of *N. laevis*. A



**Figure 4.** Effect of ethanol extracts of *Newbouldia laevis* leaves and stem on serum catalase activity. Group 1: Control (Normal rats), Group 2: Positive control (Diabetic untreated rats), Group 3: Alloxan induced diabetic rats + 2.5 mg/kg body weight of Glibenclamide, Group 4: Alloxan induced diabetic rats + 200 mg/kg body weight of ethanol leaves extract, Group 5: Alloxan induced diabetic rats + 400 mg/kg body weight of ethanol leaves extract, Group 6: Alloxan induced diabetic rats + 200 mg/kg body weight of ethanol stem extract, and Group 7: Alloxan induced diabetic rats + 400 mg/kg body weight of ethanol stem extract.

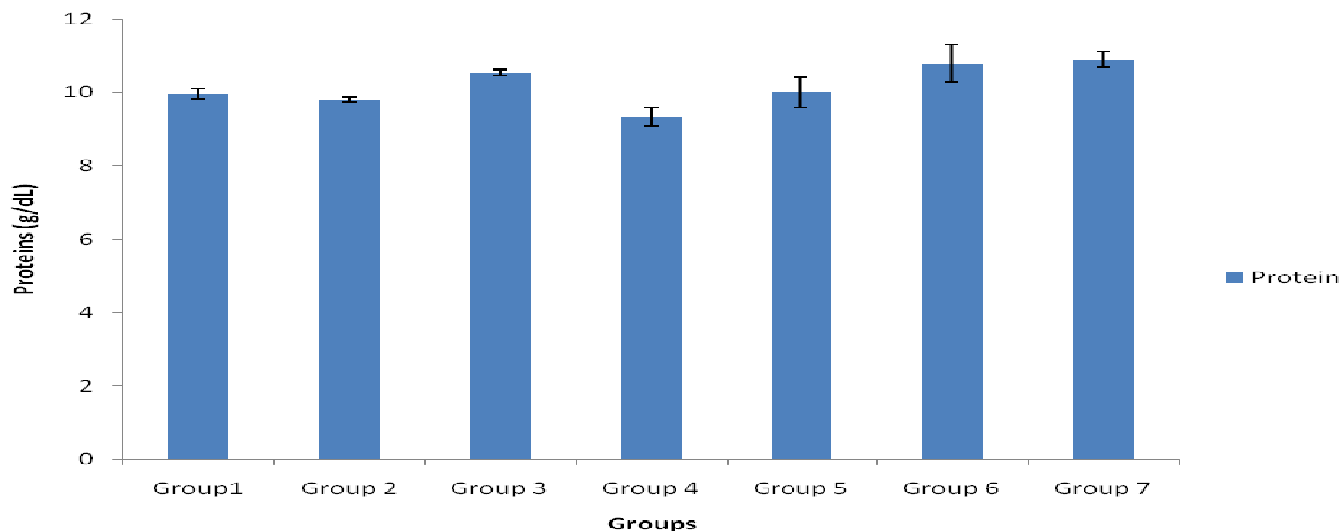


**Figure 5.** Effect of ethanol extracts of *Newbouldia laevis* leaves and stem on serum glutathione. Group 1: Control (Normal rats), Group 2: Positive control (Diabetic untreated rats), Group 3: Alloxan induced diabetic rats + 2.5 mg/kg body weight of Glibenclamide, Group 4: Alloxan induced diabetic rats + 200 mg/kg body weight of ethanol leaves extract, Group 5: Alloxan induced diabetic rats + 400 mg/kg body weight of ethanol leaves extract, Group 6: Alloxan induced diabetic rats + 200 mg/kg body weight of ethanol stem extract, and Group 7: Alloxan induced diabetic rats + 400 mg/kg body weight of ethanol stem extract.

significant increase ( $p < 0.05$ ) was observed in the catalase activity of rats in the test groups when compared with the control. A significant increases ( $p < 0.05$ ) in the serum catalase activity were observed in groups 4 and 5 (treated with the leave extract) when compared with groups 6 and 7 (treated with the stem extract).

#### Effect of ethanol extracts of *Newbouldia laevis* leaves and stem on serum glutathione

Figure 5 shows that the diabetic untreated group (group 2) had decreased glutathione but the administration of the different doses of the extracts resulted in significant



**Figure 6.** The effect of ethanol extracts of *Newbouldia laevis* leaves and stem on serum protein. Group 1. Control (Normal rats), Group 2. Positive control (Diabetic untreated rats), Group 3: Alloxan induced diabetic rats + 2.5 mg/kg body weight of Glibenclamide, Group 4. Alloxan induced diabetic rats + 200 mg/kg body weight of ethanol leaves extract, Group 5: Alloxan induced diabetic rats + 400 mg/kg body weight of ethanol leaves extract, Group 6: Alloxan induced diabetic rats + 200 mg/kg body weight of ethanol stem extract, and Group 7: Alloxan induced diabetic rats + 400 mg/kg body weight of ethanol stem extract.

( $p < 0.05$ ) increase in glutathione (GSH) level in the treatment groups when compared with the positive control (group 2). The GSH helps to mop up oxidative stress in the test groups as a result of lipid peroxidation. A significant increase ( $p < 0.05$ ) was observed in the GSH of rats in the test groups, however, no significant difference ( $p > 0.05$ ) was observed in the GSH of rats in group 4 when compared with the positive control. The increase was dose dependent even as the rats treated with the stem extracts (groups 6 and 7) expressed higher level of GSH at both doses tested when compared with groups 4 and 5 (leave extract treated).

#### Effect of ethanol extracts of *Newbouldia laevis* leaves and stem on serum protein

Figure 6 reveals that increase in protein levels in the treatment groups as against the controls appears to be dose dependent, with group 6 and 7 expressing higher increases in protein levels.

## DISCUSSION

Anti-diabetic properties of *N. laevis* leaf and stem have earlier been reported (Anaduaka et al., 2013). Increase in lipid peroxidation during diabetes may be due to the inefficient or overwhelmed antioxidant system due to free radical generation prevalent in diabetic conditions. The status of lipid peroxidation as well as altered levels of certain endogenous radical scavenger is taken as direct

evidence for oxidative stress (Goldstein et al., 1993). The liver is an important insulin dependent tissue which plays a pivotal role in glucose and lipid homeostasis and is severely affected during diabetes (Seifter and England, 1982). In this study, alloxan administration to experimental rats caused a marked elevation in the levels of serum ALP and ALT which is indicative of hepatocellular damage. This might possibly be due to the release of these enzymes from the cytoplasm into the blood circulation rapidly after rupture of the plasma membrane and cellular damage.

Several studies have reported similar elevation in the activities of serum ALP and ALT during alloxan administration (Etuk and Muhammed, 2010; Gometi et al., 2014). The notable reduction in serum ALP activity recorded is suggestive of cellular membrane/hepatocellular membrane protective effects of the extracts. ALP functions as a biochemical marker enzyme for maintaining membrane integrity. Increase in its plasma activity indicates peroxidation of cell membrane integrity. Treatment with *N. laevis* leaves and stem extracts significantly reduced the activities of these liver marker enzymes in alloxan induced oxidative stress as presented in this finding. This indicates that the extracts tend to prevent liver damage by maintaining the integrity of the plasma membrane thereby suppressing the leakage of the enzymes through the membrane, exhibiting hepatoprotective activity. A number of scientific reports indicate that certain flavonoids, terpenoids and steroids have protective effect on liver due to their antioxidant properties (Jeruto et al., 2011). Flavonoids have been reported to possess antioxidant activity (Jeruto et

al., 2011) and thus, are capable of protecting cell membranes from peroxidative actions of free radicals.

Free radical scavenging enzymes like superoxide dismutase (SOD) and catalase protect the biological system from oxidative stress (Conn, 1995; Switala and Loewen, 2002; Del-Rio et al., 2005; Waggiallah and Alzohairy, 2011). The decrease in the activity of the enzymes in the present study could be attributed to the excessive utilization of these enzymes in attenuating the free radicals generated during the metabolism of alloxan. Similar reports have shown an elevation in the status of lipid peroxidation in the liver after alloxan induction (Szkudelski et al., 1998; Gometi et al., 2013) and our findings are in accordance with these reports. Restoration in the levels of the antioxidant enzymes as shown in the above results after 21 days of treatment could be related to its ability to scavenge reactive oxygen species, thus preventing further damage to membrane lipids. Therefore, the antioxidant properties of the extracts may have resulted in the recoupment in the activities of the enzymic antioxidants (SOD and catalase).

Non-enzymic antioxidants acts synergistically to scavenge the free radicals formed in the biological system. After 21 days of treatment, the extracts effectively restored the depleted level of this non-enzymic antioxidant caused by alloxan induction.

Increase in GSH level in turn contributes to the recycling of other antioxidants such as vitamin C and E (Exner et al., 2000). This may suggest that the extracts maintain the level of antioxidant vitamins by maintaining GSH homeostasis thereby protecting the cells from further oxidative damage.

There was no significant difference ( $p > 0.05$ ) in serum protein as shown in Figure 6 when compared to the controls (group 1 and 2). This might be as a result of some proteins forming intra-chains or inter-chains disulfide bridges between cysteine residues.

The cross-links in this way help to protect the native conformation of the protein molecule from the intensity of oxidative stress (David and Cox, 2005; Biswas et al., 2006).

## Conclusion

The experiment evidence obtained in the present laboratory animal study indicates that the ethanol extracts of the leaves and stem of *N. laevis* possess hepatoprotective properties for curbing oxidative stress complications. The efficacy of the extracts can be attributed to the presence of biologically active components which may be worth further investigation and elucidation.

## Conflict of Interests

The author(s) have not declared any conflict of interests.

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