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Effect of Methanolic Leaf Extract of *Ocimum gratissimum* (Linn) Leaves on Sodium Arsenite-Induced Toxicity in Rats

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ABSTRACT

Natural plant products are considered as possible protective agents against arsenite induced toxicity. Effects of methanolic leaf extract of *Ocimum gratissimum* were investigated in sodium arsenite exposed rats. Animals were randomly divided into four groups of five per group. Group A (control), Group B (sodium arsenite alone), Group C (extract and sodium arsenite) and Group D (extract only). Rats were orally pretreated with 100 mg kg^{-1} b.wt. extract for 14 days while 2.5 mg kg^{-1} b.wt. arsenite was administered intraperitoneally on the 14th day and animals were sacrificed after 24 h. Plasma Alanine Amino Transferase (ALT), Alkaline Phosphatase (ALP) and Gamma Glutamyl Transferase (GGT) were evaluated. Hepatic lipid peroxidation as Malondialdehyde (MDA) and reduced Glutathione (GSH) levels were also assessed. Introduction of sodium arsenite in rats triggered significant increases in plasma ALT, ALP and GGT ($p<0.05$) levels. Significant increase ($p<0.05$) in hepatic MDA concentration and depletion in GSH level were obtained. The methanolic leaf extract with arsenite modulated the activities of ALT, ALP and GGT to their normal levels. The extract reversed sodium arsenite-induced decrease in hepatic GSH to their normal levels while significant effect on hepatic MDA level was not observed. Group treated with the extract alone showed no adverse effects on the parameters. The properties exhibited by the extract may be due to the presence of certain bioactive compounds in it. The results obtained from this study underpin the importance of further research to obtain bioactive substance from the leaf of *Ocimum gratissimum*.

Key words: *Ocimum gratissimum*, sodium arsenite, malondialdehyde, reduced glutathione

INTRODUCTION

Medicinal plants have been identified from indigenous pharmacopeias which have significant healing power (Holets *et al*., 2003; Kayode and Kayode, 2011). Its use against various ailments has gained widespread acceptance in developing as well as developed nations (Kolawole *et al*., 2011). *Ocimum gratissimum* (Linn) also known as "alfavaca" is a perennial herb widely distributed in tropical and warm temperate region in some African countries. It is put to use in folklore medicines to treat different ailments such as upper respiratory tract infections, diarrhea, headache, fever, skin diseases and pneumonia (Onajobi, 1986; Ilori *et al*., 1996).

In Nigeria *Ocimum gratissimum* commonly known as "Efinrin" by the Yoruba of South Western Nigeria. It is used as spices in foods and medicines while the Ibos of the Southern Nigeria use it to clean wound surface of the baby's placental cord.

Arsenic as trivalent arsenite (As^{3+}) or pentavalent arsenite As^{5+} is naturally occurring and ubiquitously present in the environment, where it is the main constituent of more than 2000 mineral species on earth (Jana *et al.*, 2006). Soluble arsenic salts such as sodium arsenite, are well absorbed after ingestion and inhalation through the gastrointestinal and respiratory tracts respectively. Absorption through percutaneous is clinically significant only after heavy exposure to arsenic reagent (Katzung, 2001). Sodium Arsenite have been shown to elicit free radicals and oxidants generation as chromosomal mutation and oxidative DNA damage have been associated with some arsenite linked human diseases (Hei, 2001). One of the mechanisms of arsenite toxicity is the elevation of cell's reactive oxygen species. The increased ROS has been shown to cause oxidative DNA damage (Hei, 2001).

Studies have established the efficacy of green plant as herbs in the treatment and/or prevention of diseases associated with hepatic and oxidative damage. However, despite multiplicity of application of *Ocimum gratissimum* in popular folk medicine, there has been surprisingly little pharmacological investigation on this plant while only a preliminary report has indicated a possible nociceptive property of its aqueous extract in Nigeria (Rabelo *et al.*, 2003). Therefore, the present study was carried out to assess the possible roles of the methanolic leaf extract of *Ocimum gratissimum* in rats exposed to sodium arsenite.

MATERIALS AND METHODS

Chemicals: Sodium arsenite ($NaAsO_2$), Thiobarbituric Acid (TBA), reduced Glutathione (GSH), Ellman's reagent were purchased from Sigma Chemical Company St. Louis, MO, USA. All other chemicals used in the experiment were of analytical grade.

Plant collection: The plant sample (*Ocimum gratissimum* leaves) was collected from the Forestry Section of the Department of Natural Products and Traditional Medicine at the National Institute for Pharmaceutical Research and Development (NIPRD) Idu, Abuja, Nigeria. The leaves were identified and confirmed and a Voucher number 5942 deposited at the herbarium section of the Institute.

Preparation of methanolic leaf extract of *Ocimum gratissimum*: Fresh leaves from the plant were cut into pieces air dried at room temperature and subjected to cold methanolic extraction. The dried leaves (200 g) were soaked in methanol (1.5 L) for 72 h after which the filtrate was allowed to dry at room temperature.

Animals: Twenty four male Wistar Albino rats obtained from the animal house of the Department of Anatomy, Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology Ogbomoso, Nigeria were used for this experiment. All animals were fed on rats pellets (obtained from Ladokun Feeds Limited, Ibadan) and maintained under Laboratory conditions for two weeks prior to commencement of the experiment. They were allowed food and water *ad libitum* before and during the experiment.

Experimental protocol: Using corn-oil as a vehicle, 100 mg kg^{-1} b.wt. extract was made into 0.1 mL corn oil and given to the rats once daily for seven days by intubation. Sodium arsenite (2.5 mg kg^{-1} b.wt. made into 0.1 mL distilled water) was given intraperitoneally only once on the seventh day to animals in group B and C after which all the animals were fasted and sacrificed 24 h after administration of sodium arsenite (Ramos *et al.*, 1995) through cervical dislocation. The groups and the agents administered are shown as:

Group A: Control rats received only saline solution
Group B: Received saline and sodium arsenite on the 7th day only
Group C: Received extract and sodium arsenite once on the 7th day
Group D: Rats received only the extract for 7 days

All procedures and steps were carried out between 0-4°C. The rat liver was excised and homogenized in homogenizing buffer (w/v: 1/4) using Teflon head homogenizer. The homogenate was centrifuged at 9000 rpm for 10 min at 4°C and the supernatant was collected.

Blood plasma was prepared from the blood sample obtained from the rat heart via cardiac puncture into EDTA vial. This was centrifuged at 3000 rpm for 10 min and the plasma (supernatant) collected using a Pasteur pipette into fresh sample bottles.

Biochemical evaluation: Tissues and plasma proteins were estimated based on the principle of the Biuret reaction (Gornall *et al*., 1949). ALT activity was determined by the method of Reitman and Frankel (1957). ALP was determined by using the method described by Klein *et al.* (1960). GGT activity was determined by the method of Szasz (1969).

Assessment of lipid peroxidation was carried out by the method of Varshney and Kale (1990). Estimation of the levels of hepatic reduced glutathione (GSH) was carried out by the method of Beutler *et al.* (1963).

Statistical analysis: Data were presented as Mean±SEM from 6 animals in each group. Results were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for comparisons of different means using SPSS statistical software. Statistical significance was set at $p<0.05$.

RESULTS

Effects of *Ocimum gratissimum* on plasma ALT and ALP activities are shown in Fig. 1 and 2, respectively. Plasma ALT and ALP activities were significantly ($p<0.05$) elevated in the sodium

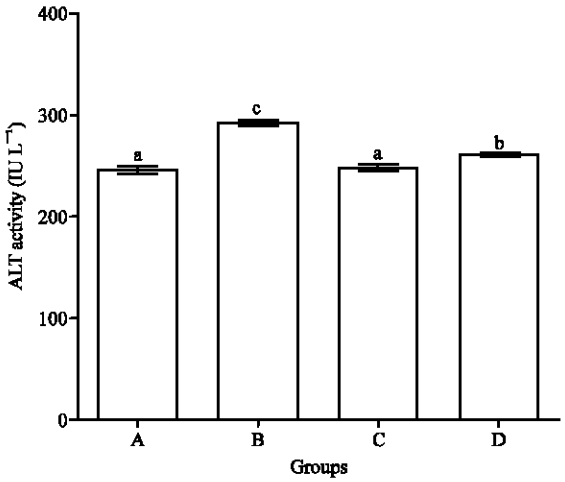

Fig. 1: Effect of methanolic leaf extract of *Ocimum gratissimum* on plasma ALT activities of rats. Each value is Mean±SE of 6 rats in each group. Bars with different alphabets are significantly different from each other at $p<0.05$

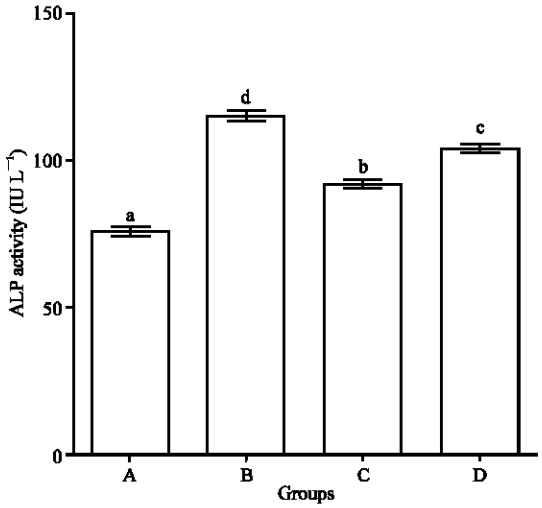

Fig. 2: Effect of methanolic leaf extract of *Ocimum gratissimum* on plasma ALP activities. Each value is Mean±SE of 6 rats in each group. Bars with different alphabets are significantly different from each other at $p<0.05$

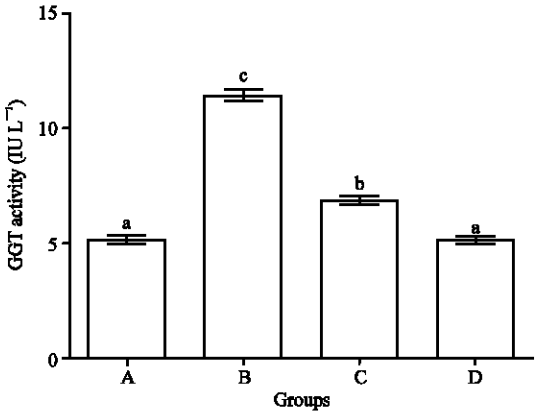

Fig. 3: Effect of methanolic leaf extract of *Ocimum gratissimum* on plasma GGT activities. Each value is Mean±SE of 6 rats in each group. Bars with different alphabets are significantly different from each other at $p<0.05$

arsenite group. Co-administration of methanolic leaves extract of *Ocimum gratissimum* with sodium arsenite significantly lowered the activities of both ALT and ALP ($p<0.05$).

Sodium arsenite significantly increased GGT activity while co-administration of the extract with sodium arsenite drastically lowered the enzyme activity ($p<0.05$). The extract administered alone did not show any effect on the activity of plasma GGT (Fig. 3).

Sodium arsenite induced increase in the level of liver MDA as shown in Fig. 4. Co-administration of the extract with sodium arsenite did not restore the liver MDA concentration back to normal level.

The hepatic GSH concentration in Sodium arsenite-treated animals was significantly lower than that of control ($p<0.05$) (Fig. 5). In contrast, the hepatic GSH levels of group treated with sodium arsenite and the extract was similar to control level and significantly higher than that of rats treated with sodium arsenite alone ($p<0.05$).

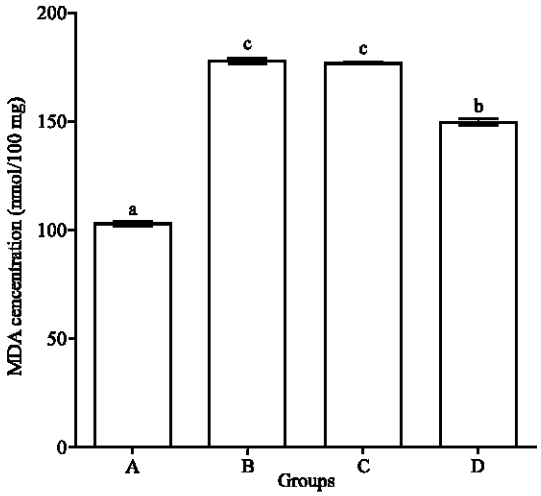

Fig. 4: Effect of methanolic leaf extract of *Ocimum gratissimum* on hepatic MDA concentrations. Each value is Mean±SE of 6 rats in each group. Bars with different alphabets are significantly different from each other at $p<0.05$

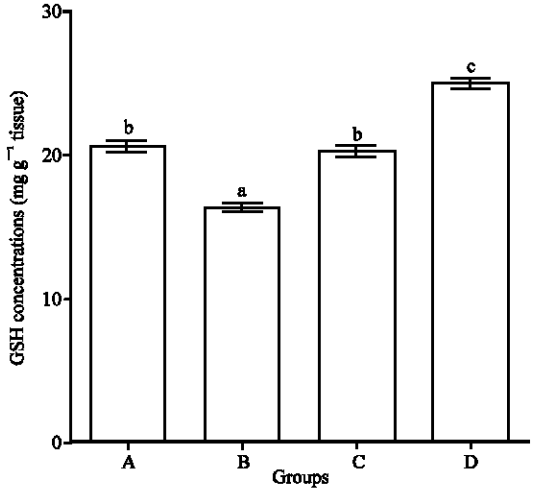

Fig. 5: Effect of methanolic leaf extract of *Ocimum gratissimum* on hepatic GSH concentrations. Each value is Mean±SE of 6 rats in each group. Bars with different alphabets are significantly different from each other at $p<0.05$

DISCUSSION

Plasma ALT, ALP and GGT are essential enzymes in biological processes. These enzymes are considered as biomarkers of liver injury (Schieg, 1996). Elevated levels of these enzymes are associated with several pathologies such as myocardial infarction, liver cirrhosis, hepatitis and neoplasm (Krupp *et al*., 1987). The present study shows that sodium arsenite induced increase in these enzymes. Administration of the extract in rats exposed to sodium arsenite ameliorated the effect of the toxicant (Fig. 1-3). This suggests modulatory role of the extract against sodium arsenite-induced hepatotoxicity (Dajas *et al*., 2005; Ijeh *et al*., 2005) which could be attributed to prevention of intracellular leakage of the enzymes (ALT, ALP and GGT) via membrane stability (Balouchzadeh *et al*., 2011). However, rats administered with the extract alone showed increases

in the activities of these enzymes, although the increases were not significant compared with the rats exposed to sodium arsenite alone. This indicates that the use of this plant for folklore treatment should be with guide.

The constituents of cell biomembrane are liable to free radical and active oxygen damage. The actions of these free radicals trigger cell damage by covalently binding with cellular macromolecules and formation of lipid peroxidation (Ajay *et al*., 2010) which are implicated in processes such as carcinogenesis, inflammation and aging (Ames *et al*., 1993). Arsenite has been shown to exert its harmful effect by increasing the cell's production of free radicals (Hei *et al*., 1998) which, eventually lead to increase in lipid peroxidation with attendant production of MDA and lipid peroxide (Flora, 1999). Lipid peroxidation causes increase oxidative stress (Diallo *et al*., 2009), alters membrane structural composition leading to malfunction of membrane bound enzymes and overall decrease of cellular functions (Bashandy and Alwasel, 2011). The result as presented in Fig. 4 shows that sodium arsenite induced significant increase in hepatic MDA. The treatment with the extract did not significantly lower the induced increase in hepatic MDA.

GSH functions as a scavenger of ROS such as $OH^{\bullet}$, singlet oxygen, nitric oxide and peroxynitrite (Halliwell and Gutteridge, 1989). The levels of GSH in cells are very important to deteriorative effect of toxic substances (Balouchzadeh *et al*., 2011). A decrease in cellular GSH level has been inversely correlated with lipid peroxidation (Maiti and Chatterjee, 2001; Elsaid *et al*., 2011). This correlates with the result obtained in the present study. The result in Fig. 5 shows that sodium arsenite lowered the hepatic GSH. Treatment of rats with the extract protected the liver by reversing sodium arsenite-induced decrease in hepatic GSH level. This implies that the extract has antioxidative property and probably has ability to lower the binding affinity of sodium arsenite with sulphridyl group of GSH.

CONCLUSION

The results of this study show that methanolic leaf extract of *Ocimum gratissimum* may contain certain bioactive agents with therapeutics property which may give credence to its wide medicinal use across West African countries. However, detailed studies need to be carried out to characterized and isolate its active compounds as possible template in drug discovery.

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