

Toxicity of the Concurrent Administration of the Ethanol Leaf Extract of African Eggplant and Bitter Leaf on the Stomach and Selected Haematological Ratios in Adult Male Wistar Rats

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ABSTRACT

Background: African eggplant and bitter leaf plant are important plants in many African communities; they are eaten as vegetable and also used in the management of metabolic disorders. This study investigated the sub-acute effect of oral administration of ethanol extract of African eggplant and bitter leaf plant on selected haematological ratios; platelets to lymphocyte ratio (PLR) and neutrophil to lymphocyte ratio (NLR), the histology and antioxidant enzymes activity Catalase (CAT), Malodialdehyde (MDA), reduced Glutathione (GSH) and Superoxide dismutase (SOD) in the stomach of male Wistar rats. **Methodology:** The rats were grouped into four; control [A] which received no treatment and 3 test groups; B [100 mg/kg of African eggplant], C [100 mg/kg of bitter leaf], and D [100 mg/kg of bitter leaf and 100 mg/kg of African eggplant] respectively, orally administered for 14 days. **Results:** Results revealed that group D rats showed significant changes in the level of SOD, GSH and MDA activities alongside significant changes in some antioxidant enzymes levels of the stomach of groups B and C rats. The histopathology review of the stomach across all test groups showed some pathological changes. Across all test group there was an increase in PL- ratio and NL- ratio when compared to the control. **Conclusion:** Extrapolating from the results of this study, the 1000 mg/kg ethanol leaf extract of African eggplant and bitter leaf concurrently is toxic to the stomach and may also cause inflammation and should be consumed with caution.

Keyword: African eggplant, antioxidant, bitter leaf plant, hematological ratios, stomach

INTRODUCTION

The use of medicinal plant in recent times has become high, this is due to the fact that they are cheap, available, and potent with have little or

no side effects ^[1]. In many communities in Africa, traditional medicine that involves the use of medicinal plant in the treatment of disease and

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infection makes up major aspect of their health care system. Plants that contain bioactive compounds that can cause physiological changes that may either be beneficial or toxic to the body system are called medicinal plants. These bioactive compounds are secondary metabolites in plant. They form the plant defense system and are also responsible for the coloration of the plant. In the body system, these compounds play role in the protective or toxic function and they include flavonoids, phenols, glycoside, saponin, steroid and alkaloid^[2]. According to different experimental studies, the presence of these compounds in plant has conferred on them the ability to exert; antidiabetic activity^[3], anti-microbial activity^[4], hepato-protective effect^[5], nephro-protective effect^[6] and gastro-protective effects^[7].

African eggplant, also known as *Solanum macrocarpon L.* and bitter leaf plant, known as *Vernonia amygdalina Del* are two major vegetables in tropical Africa that are consumed and used in traditional medicine. The fruit of African eggplant are used to treat stomach problem, heart disease and cardiovascular conditions while the leaves are used in the treatment of parasitic infections^[8]. It is also acclaimed to reduce the risk of diabetes and arteriosclerosis^[9]. Bitter leaf plant widely consumed in tropical Africa, is believed in traditional medicine to treat fever, hiccups, renal issues and stomachaches^[10]. In experimental animals, pharmacological studies have indicated that the leaf extracts have both anti-hyperglycemic and hypolipidaemic activities, suggesting that they could be utilized to manage and treat diabetes^[11].

Plants use for medicinal purposes are often thought to be safe, however they do have some harmful effects and toxicities^[12]. When medicinal plants are used in a specific form, at larger amounts, or in conjunction with other plants or medications, they can be hazardous. Due to these reasons, this study evaluates the toxic effect of the ethanol leaf extract of African eggplant and bitter leaf on the histology,

antioxidant enzymes activity, weight of the stomach and selected hematological ratios of male Wistar rats.

MATERIAL AND METHODS

Animal Care

Twenty adult healthy male Wistar rats weighing between 160 g – 200 g were used for this experiment. They were gotten from parent stock in the animal house of the Obafemi Awolowo College of Health Science, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria and kept in plastic and wire gauze cages. The rats were maintained on standard rat chow from Joyful Feeds and Flour Mills Ltd, Nigeria. Food and water were provided *ad libitum*. The care and handling of the animals were in accordance with the internationally accepted standard guidelines for animals' use by the National Research Council (2011)^[13].

Please state your ethical considerations and provide evidence of approval from your institutional animal care and use committee (IACUC) or its equivalent.

Animal Grouping: The animals were randomly assigned in to four groups: A, B, C, and D with five rats in each group. Group A rats consisted of normal rats which were fed with feed and water only (control group), group B rats were administered with 1000 mg/kg BW of African eggplant ethanol leaf extract, group C was administered with 1000 mg/kg BW of bitter leaf ethanol leaf extract, and group D was administered with the concurrent administration of 1000 mg/kg BW of bitter leaf plant in the morning and 100 mg/kg BW Africa eggplant ethanol leaves extract in the afternoon.

Plants Material

Matured leaves of bitter leaf plant (*Vernonia amygdalina Del*) were collected from an indigenous farm in Ikenne/Sagamu area of South West, Nigeria, while matured leaves of African eggplant (*Solanum macrocarpon L.*) were bought from a local market in Ikenne/Sagamu area of

South West, Nigeria.

Preparation of the Ethanol Extract of African Eggplant and Bitter Leaf Plant

Leaves of African eggplant and bitter leaf plant were air dried and powdered with the use of a blender. Weighed powdered form of 150 g was soaked in 750 ml of ethanol [70% ethanol and 30% water] for 3 days inside a refrigerator. The resultant solution was filtered using a funnel plugged with glass wool. The filtrate was heated at a temperature of 40°C for 5 minutes to allow ethanol to evaporate and the residue was kept for further use.

Administration of Plant Extract

Ethanol extract of the leaves of African eggplant and bitter leaf plant [1000 mg/kg BW] were administered orally to the rats using oral cannula. Treatment was done in the morning every day before the animals were fed and, in the afternoon for the co-administration group, for a period of two weeks [14 days].

Determination of Organ Weight

After the last administration, the animals were fasted overnight and sacrificed via cervical dislocation. Following a midline abdominal incision, the stomach was harvested. After the stomachs were excised, they were weighed using a scale.

Histological Examination

The stomach of all the rats were harvested and fixed in 10% neutral buffered formalin, embedded in paraffin, and 5 µm thick sections were prepared and stained with Haematoxylin and Eosin using standard laboratory procedures. Photomicrographs were taken using a Leica DM750 Camera Microscope at x400 magnification after viewing the slides under a light microscope.

Procedure for Determination of Antioxidant Enzymes

The stomach tissue assessed for oxidative studies were homogenized in phosphate buffer in ratio four

to one. Superoxide dismutase [SOD], glutathione reductase [GSH], catalase [CAT] and malondialdehyde [marker of lipid peroxidation [MDA] activities were determined. Superoxide dismutase activities were determined according to the method of Valerino and McCormack^[14]. Increased absorbance was monitored with a UV spectrophotometer at 480 nm every 60 seconds for 180 seconds. SOD activity was defined as the amount of SOD needed to inhibit the oxidation of adrenaline to adrenochrome by 50% for one minute. The activity of SOD was expressed as µg/mg protein. Reduced glutathione was determined using the methods of Sedlak and Lindsay^[15]. The absorbance of the yellow color formed upon the addition of Ellman's reagent was read within 5 minutes at 412 nm with a UV spectrophotometer. A plot of absorbance versus concentration of reduced GSH was then obtained from a serial dilution of the stock GSH prepared by adding 1.5 mL of phosphate buffer and 1.5mL of Ellman's reagent^[16]. The amount of GSH was expressed as µg/mg protein. Catalase activity was determined with the method described by Shina^[17]. Proper dilution of the serum samples was done at ratio one to ten dilution in series. Catalase was expressed as millimoles of H₂O₂ consumed per minute per mg protein. It used the principle that dichromate in acetic acid is an unstable intermediate. The chromic acetate produced was measured colorimetrically at 570 nm.

Malondialdehyde [MDA] was determined spectrophotometrically from the pink color product of thiobarbituric acid [TBA] reactive substances complex. The test sample was mixed with 0.1mL of 10% TCA, followed by 0.5mL of 75% TBA. The mixture was then placed in a water bath at 80 degrees Celsius for 45 minutes. The resulting pink solution's absorbance was measured against a reference blank of distilled water at 532nm. The test sample was calibrated using the MDA as a standard and the result was expressed as the amount of free MDA produced. The MDA level was calculated according to Adam-Vizi and Sergi,^[18]

The Lipid peroxidation was expressed as $\mu\text{g}/\text{mg}$ protein.

Procedure for Blood Collection and Hematologic Analysis

Blood was collected from the orbital venous sinus, the rat was restrained, the neck gently scruffed and the eye made to bulge. A capillary tube was inserted medially into the eye and blood was allowed to flow by capillary action through the capillary tube into EDTA sample bottle^[19].

White blood cell count [WBC], percentage lymphocyte, percentage neutrophils, monocyte, eosinophil, hemoglobin concentration [HGB], and platelet count [PLT] were estimated using an automated Beckman coulter auto-analyser according to the method of Kakei^[120]. neutrophil to lymphocyte ratio [NLR], platelet to lymphocyte ratio [PLR], was calculated by the methods described by Aly *et al.*,^[21].

The statistical analysis of data collected from this study was done using the SPSS-8.0 statistical software package. The data was presented as Mean \pm Standard Error of Mean [SEM] and statistical analysis was carried out using the student t-test and Analysis of variance [ANOVA]. Values were considered to be statistically significant when $p < 0.05$.

RESULTS

Effect of the oral administration of the ethanol extract of African eggplant and bitter leaf on the histology of the stomach

The co-administration treatment group showed degenerative effect of ethanol extract of African eggplant and bitter leaf on the histology of the stomach. In groups treated with 1000 mg/kg of bitter leaf and 1000 mg/kg of African eggplant, there was distortion in the mucosa of the stomach.

Figure 1a-d; Photomicrograph of stomach tissue following administration of African eggplant only, bitter leaf only and a co-administration of both. [H/E $\times 400$]

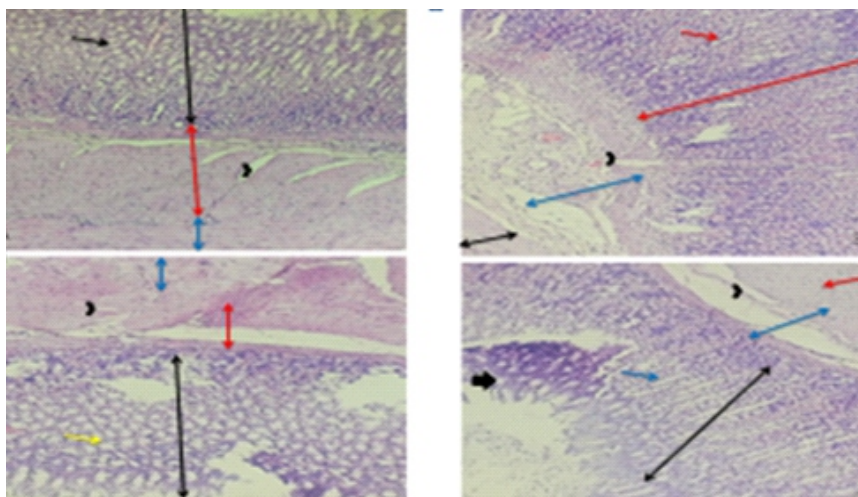
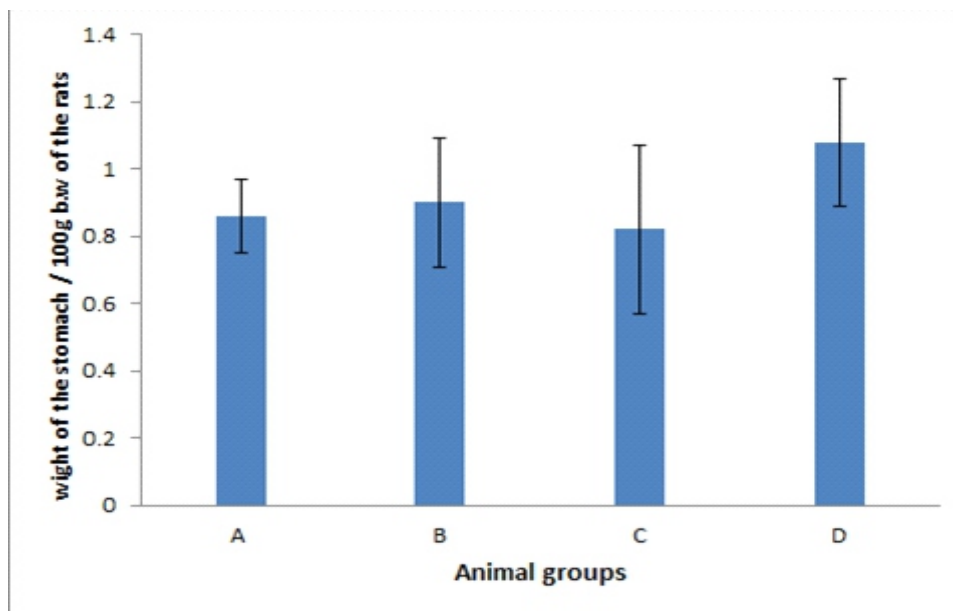


Figure 1a [control group], mucosa [black double arrow head], sub mucosa [red double arrow head], muscularis external [blue double arrow head], arterioles [black arrow head] and gastric glands [black thin arrow]. **Figure 1b [1000 mg/kg of African eggplant]**, the mucosa [red double arrow head] with slight distortion, sub mucosa [blue double arrow head], the muscularis external [black double arrow head] and the arterioles [black arrow head] are well differentiated; the gastric glands [black thin arrow] are constricted and narrowed. **Figure 1c [1000 mg/kg of bitter leaf plant]**, slight distortion at the mucosa layer [black double head arrow], the submucosa [red double head arrow], muscularis external [blue double head arrow], gastric glands [yellow thin arrow] and arterioles [black arrow head] are well defined. **Figure 1d [1000 mg/kg of African eggplant and 1000 mg/kg of bitter leaf]**, shows necrotic mucosa layer [black thick arrow], constricted and narrowed gastric glands [blue thin arrow], dilated arterioles [black arrow head], the sub mucosa [blue double head arrow], and muscularis external [red double head arrow] are well differentiated.

Effect of the oral administration of the ethanol extract of African eggplant and bitter leaf on the weight of the stomach

As shown in figure 2, only group C has a lower relative stomach weight when compared with the control group and other test groups.



A: control group, B: 1000 mg/kg African eggplant, C: 1000 mg/kg bitter leaf plant, D: 1000 mg/kg of African eggplant and 1000 mg/kg of bitter leaf plant

Figure 2: Chart showing relationship among the relative stomach weights in the different administrations of African eggplant only, bitter leaf only and a combination of both.

Effect of oral administration of ethanol extract of African eggplant and bitter leaf on antioxidant enzymes activity of the stomach

When compared to the control group, result from Table 1 shows significant changes [$P < 0.05$] in antioxidant enzymes activity in the stomach of male Wistar rats with the administration of ethanol leaf extract of African eggplant and bitter leaf plant. GSH activity increased significantly across all treatment groups when compared to group A. SOD activity of the stomach of group B and C rats significantly decrease while group D rats showed significant increase when compared with the control group. Only group C showed significant reduction in CAT activity when compared with the control group. There was significant increase across all groups in the level of MDA when compared with the control group

Effect of oral administration of ethanol extract of African eggplant and bitter leaf on antioxidant enzymes activity of the stomach

When compared to the control group, result from Table 2 shows significant changes [$P < 0.05$] in the haematological ratios of male wistar rats administered with the ethanol leaf extract of African eggplant and bitter leaf plant. NLR value increased across all test groups but this increase was not significant when compared to the control group. PLR showed statistical significant increase across all test group when compared to the control group.

Table 1; Effect of administration of Ethanol leaf extract of African Eggplant and Bitter leaf on the antioxidant enzymes activity of the stomach in male Wistar rats

Grps	Treatment	GSH[$\mu\text{mol/ml}$]	SOD [$\mu\text{mol/ml/min/mg pro}$]	CAT [$\mu\text{mol/ml/min/mg pro}$]	MDA [$\mu\text{mol/ml}$]
A	Control	13.75 \pm 1.39	13.56 \pm 1.66	14.52 \pm 3.42	1.86 \pm 0.67
B	African eggplant	23.34 \pm 4.92 ^a	9.39 \pm 0.76 ^a	11.71 \pm 2.03	0.58 \pm 0.41 ^a
C	Bitter leaf plant	16.82 \pm 1.01 ^a	6.40 \pm 1.99 ^a	11.24 \pm 0.42 ^a	3.59 \pm 1.45 ^a
D	Bitter leaf plant and Africa eggplant	15.04 \pm 1.99 ^a	15.87 \pm 1.5 ^a	18.03 \pm 2.85	2.66 \pm 0.38 ^a

Each value is an expression of mean \pm SEM. [P < 0.05]

^a - Values were significant when compared to group A

Table 2: Effect of oral administration of ethanol extract of African Eggplant and bitter leaf on antioxidant enzymes activity of the stomach

Grps	Treatment	NLR	PLR
A	Control	0.59 \pm 0.17	14.64 \pm 3.72
B	African eggplant	0.86 \pm 0.30	24.03 \pm 4.61 ^a
C	Bitter leaf plant	0.80 \pm 0.70	20.88 \pm 6.29 ^a
D	Bitter leaf plant and Africa eggplant	1.16 \pm 0.91	29.91 \pm 12.66 ^a

Each value is an expression of mean \pm SEM. [P < 0.05]

^a - Values were significant when compared to group A

DISCUSSION

The gastrointestinal tract [GIT] is the first organ that directly comes in contact with orally introduced substances. Thus, potentially toxic substances taken orally may damage the GIT [22]. Since adequate morphological investigations are useful for the anatomical localization of toxin action, functional toxicology research should be paired with appropriate histology studies [23]. In the histology of the stomach of the rats used in this study, group D rats showed dilatation of the arteries and necrotic mucosal layer. Gastric necrosis was also seen which may be due to intra-thoracic herniation, volvulus, acute necrotizing gastritis, drinking corrosive substances, and vascular compression [24, 25]. Since gastric necrosis is not found in the control group or other test group, the co-administration of African eggplant and bitter leaf is corrosive to the mucosa of the stomach and this may even be responsible for the increase in

weight of the stomach. The mechanism of lipid peroxidation is triggered by free radicals. Malondialdehyde [MDA] is a result of the breakdown of polyunsaturated fatty acids in cells. Overproduction of MDA is caused by an increase in free radicals. The quantity of malondialdehyde is a frequent indicator of oxidative stress and antioxidant status [26]. In the co-administration group and 100 mg of bitter leaf treated group, there was an increase in the level of MDA, with decrease in the level of MDA in African eggplant treated group. This shows that the co-administrations group and bitter leaf plant may be toxic to the stomach by increasing the oxidative stress level in the stomach tissue.

CAT is a common antioxidant enzyme present almost in all living tissues that utilize oxygen [267] and its deficit or alteration has been connected to a

variety of diseases and abnormalities^[28,29]. In group C, the decrease in CAT activity was significant which indicate that bitter leaf may have toxic effect on the stomach tissue, the co- administration of African eggplant and bitter leaf caused an increase in the CAT activity of the stomach tissue, indicating protective activity.

Glutathione [GSH] functions as a redox moderator and is required for cellular protection as an important antioxidant. It plays a major role in removal of many reactive species^[30] and helps to detoxify cells by reacting with hydrogen peroxide and organic peroxides, protecting them from oxidative damage^[31]. In the stomach, the level of GSH in the co-administration group increased showing that the treatment has anti- GSH depletion effect on the stomach tissue^[32]. The decrease in GSH enzymes in the stomach of group B and C rats shows the accumulation of toxic material according to Iya *et al.*,^[323].

The first detoxifying enzyme and most effective antioxidant in the cell is superoxide dismutase [SOD]. It's a crucial endogenous antioxidant enzyme that is a part of the body's initial defense line from reactive oxygen species [ROS]^[27]. SOD deficiency is quite prevalent. It is critical for cellular health as it protects body cells from dangerous oxygen radicals, free radicals, and other agents that cause aging or cell death^[34]. The decrease in SOD activity shows presence of high level of free radicals as seen in test groups B and C, while test group D showed increase in SOD activity showed the co- administration of bitter leaf and African eggplant has the ability to reduce free radicals by increasing the activity of SOD enzymes in stomach.

Systemic inflammatory markers include the neutrophil to lymphocyte ratio [NLR], platelet to lymphocyte ratio [PLR], and hemoglobin to platelet ratio [HPR]. NLR is a simple, affordable, and readily available prognostic predictor^[35] it is also an accessible marker of immune response to a variety of viral and non-infectious stimuli across

practically all branches of medicine^[36]. The NL-ratio increased in all test groups, possibly indicating inflammation. The increase in NLR has been proposed as a simple indicator of the systemic inflammatory response syndrome [SIRS]^[37]. High NLR readings are related with severe inflammation, stress, injury, trauma, or major surgery, or cancer, and reflect the degree of immune-inflammatory reactivity and physiological stress to traumas or disease^[38]. The platelet lymphocyte ratio [PLR] is a new systemic inflammatory marker. A high platelet count is frequently linked to higher platelet activity^[39]. High platelet counts indicate increased thrombosis and the production of mediators that promote inflammation and atherosclerosis, such as thromboxane A2, adenosine diphosphate, serotonin, platelet activating factor, and platelet-derived growth factor. Increased platelet activity has been linked to an increase in inflammation severity^[40]. The result of this study shows a significant increase in the PL- ratio, A high PLR can be seen when platelet count is high or lymphocyte count is low^[39]. Studies have shown that a higher PLR, is associated with increased thrombosis and inflammation, which might be due to an increase in platelet activity^[41,42].

CONCLUSION

The results of this present study indicate that the co-administration of African eggplant and bitter leaf at 1000 mg/kg may has gastro-toxic effect on the stomach; it is corrosive to the mucosa of the stomach and may cause inflammation of the stomach tissue, which may also be related to the increase in lipid peroxidation [MDA]. There was increase in SOD and GSH activity of the stomach and decrease in CAT activity of the stomach tissue. The treatment of 1000 mg/kg of ethanol leaf extract of African eggplant and bitter leaf generated an increase in the NL- ratio and PL- ratio in our study, suggesting that it may be capable of creating physiological stress, which could lead to inflammation. Further studies are being carried out on effect of lower doses during short time duration

of administration.

Conflict of Interest

The authors have no conflict of interest

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