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Renal Morpho-Histological Changes and Antioxidant Enzymes Activities in Male Wistar Rats During Administration of Ethanol Leaf Extract of African Eggplant and Bitter Leaf

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ABSTRACT

This study evaluated the sub-acute effect of African eggplant, bitter leaf plant, and their combination on the antioxidant enzyme activities, histological changes, and weight of the kidney in male Wistar rats. The rats were grouped into four; control group (A) which received no treatment and 3 test groups; group B (100mg/kg BW of African eggplant), group C (100mg/kg BW of bitter leaf) and group D (100mg/kg BW of bitter leaf and 100mg/kg BW of African eggplant). The treatment was administered for 14 days. Results from this study showed that there were no observed histological changes in the kidney of groups B and C when compared to the control group but in group D there was progressive abortion of the glomeruli. For antioxidant enzyme activities across all groups, there was significant increase in the GSH activity and decrease in CAT activity in the kidney. MDA activity in groups C and D increased but decreased in group B. Groups B and D showed decrease, while group C showed increase in SOD activity. Only groups B and D showed increase in the weight of the kidney. Observations from the results showed that the administration of the two treatments, even at different time intervals caused a decrease in important antioxidant enzymes alongside increase in lipid peroxidation. Moreso, there was onset of degenerative changes in the kidney of the rats that received the two treatments. Conclusively, from our findings, co-administration of bitter leaf and African eggplant may have toxic effect on the kidney.

Keywords: African eggplant, kidney, histology, bitter leaf, antioxidant.

INTRODUCTION

Over the years, research on the effect of medicinal plants on the body system using animal models has increased and this is due to the fact that more people are now replacing the use of synthetic drugs with herbal drugs. Herbal drugs are mostly produced from medicinal plants which are known to contain phytochemicals; which are physiologically active compounds that can affect the human body. Plants use these substances as a means of self-defense, while in the human body they cause changes that may be therapeutic or toxic to the human body[1]. The presence of bioactive components in medicinal plants, which may exert intrinsic effects on cells, is directly linked to their toxicity. Contamination, adulteration and misclassification of medicinal plants are all major causes of toxicities, which are mostly caused by the presence of external harmful substances rather than the phytochemicals themselves. This accounts for the presence of toxic bioactive compounds[1].

The African eggplant (*Solanum macrocarpon*) is a tropical perennial plant in the Solanaceae family. Fruits and young leaves are the parts of the plant that are eaten. *S. macrocarpon's* roots, leaves, and fruit have been claimed to have therapeutic dietary potential[2]. It is a widely distributed plant genus in the *Solanaceae* family[3]. The leaf is an important component of the diet in Africa, since they are regarded to have a high nutrient content and are consequently utilized in soups and stews. Protein, fat, crude fiber, calcium, and zinc are all abundant in the leaf[2]. Obesity, asthma, seasonal allergies, sinus rhinitis, dermatitis, rheumatism, swelling joint pains, gastrointestinal disorders and constipation, are among the disorders treated with *S. macrocarpon* in traditional medicine[1]. The aqueous extract of the fruit has been acclaimed to lower high blood pressure, treat constipation and lower hyperlipidemia[4].

Bitter leaf plant, botanically called *Vernonia amygdalina,* is a tiny tree that can be found all over tropical Africa. The plant has been cultivated in some parts of West Africa, such as Nigeria, and is colloquially known as "bitter-leaf," while the Yoruba tribe calls it “ewuro”. The juice or extract is used as a tonic drink, and the leaves are utilized as a leafy vegetable in the popular bitter-leaf soup. In terms of dry matter, it comprises 18 percent protein, 8.5 percent fiber, and a healthy balance of macroelements and microelements[5].

Traditional medicine has utilized *V. amygdalina* as an antihelminthic, antimalarial, and laxative herb[6]. Saponins and alkaloids[7], terpenes, steroids, coumarins, flavonoids, and phenolic acids [8] are among *Vernonia amygdalina's* physiologically active chemicals.

The cells of the body act as a transport system for different substance coming into the body, this means that they are indirect recipient of these substance, therefore they are mostly affected either positively or negatively by the material they receive. Medicinal plant has both positive impact and negative impact on the cell, thereby affecting its function. This study evaluates the sub-acute effect of the administration of the ethanol extract of African eggplant and bitter leaf plant on the histomorphology, antioxidant enzymes activity and weight of the kidney.

MATERIALS AND METHODS

Animal Care: Twenty healthy, adult, male Wistar rats weighing between 160g - 200g were used for this experiment. They were gotten from parent stock in the animal house of the Obafemi Awolowo College of Health science, Sagamu campus, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria and kept in plastic and wire gauze cages. The rats were maintained on Growers feed from Joyful Feed and Flour Mill Ltd, Nigeria. Food and water were provided *ad libitum*.

Animal Grouping: The animals were randomly assigned into four groups; A, B, C, and D with five rats in each group.
Group A - normal rats, were on water (Control group)
Group B – 100 mg/kg rats of African eggplant ethanol leaves extract only
Group C – 100 mg/kg of bitter leaf plant ethanol leaves extract only
Group D – 100 mg/kg of bitter leaf plant in the morning and 100 mg/kg Africa eggplant ethanol leaves extract in the afternoon (co-treatment)

Plants Material: Matured leaves of bitter leaf plant were collected from an indigenous farm, while matured leaves of African eggplant were bought from a local market, both in Ikenne/Sagamu area of South Western Nigeria. These samples were identified and authenticated at the Department of Botany, Olabisi Onabanjo University, Ago-Iwoye, Ogun state, 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 in 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 min to allow ethanol to evaporate and the residue was kept for further use.

Adminstration of Plant Extract: Ethanol extract of the leaves of African eggplant and bitter leaf plant (100 mg/kg B.W.) were administered orally to the rats using an oral cannula. Treatment was done in the morning every day before the animals were fed and the in the afternoon for the co-treatment for a period of two weeks (14 days).

Determination of Percentage Yield of African Eggplant and Bitter Leaf Plant: The percentage of African eggplant extract was determined by calculating the percentage of the weight of the extract to the original weight of the dried sample, using the formula;

$$percentage\ yield = \frac{weight\ of\ extract}{weight\ of\ sample} \times \frac{100}{1}$$

Weight of African eggplant = 150 g
Weight of dried extract of African eggplant = 76.6 g

$$ercentage\ yield = \frac{76.6\ g}{150\ g} \times \frac{100}{1} = 51.07\%$$

The percentage yield for African eggplant is 51.07%

The percentage yield for bitter leaf plant was also calculated using the same formula stated above;

$$percentage\ yield = \frac{weight\ of\ extract}{weight\ of\ sample} \times \frac{100}{1}$$

Weight of bitter leaf plant = 150 g
Weight of dried extract of bitter leaf plant = 79.9 g

$$percentage\ yield = \frac{79.9\ g}{150\ g} \times \frac{100}{1} = 53.3\%$$

The percentage yield for bitter leaf is 53.3%

Determination of Organ Weight: The animals were sacrificed by cervical dislocation 24 hours after the last dosage. The kidneys were harvested following midline abdominal incision. The excised kidneys were weighed, respectively, using a weighing balance (kerro BL20001).

Histological Examination: The kidneys of all the rats were harvested and fixed in 10% neutral buffered formalin, processed for paraffin wax embedding, and 5 µm thick sections were cut and stained with Haematoxylin and Eosin stain using standard laboratory procedures. The slides were viewed under light microscope and photomicrographs of areas of interest were taken at x400 magnification using Leica DM750 Camera Microscope.

Procedure for Determination of Antioxidant Enzymes: The kidney tissue assessed for oxidative studies were homogenized in phosphate buffer in ratio 4:1. Superoxide dismutase (SOD), glutathione reductase (GSH), catalase (CAT) and malondialdehyde (MDA) activities were determined using standard

methods. Superoxide dismutase activities were determined according to the method of Valerino and McCormack[9]. Increased absorbance was monitored with a UV spectrophotometer at 480 nm every 60 seconds for 180 seconds. One unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline to adrenochrome during one minute. The activity of SOD was expressed as µg/mg protein. Reduced glutathione was determined using the methods of Sedlak and Lindsay [10]. 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 1.5 mL of Ellman's reagent[11]. The amount of GSH was expressed as µg/mg protein. Catalase activity was determined with the method described by Shina[12]. Proper dilution of the serum samples was done at ratio 1:10 dilution in series. Catalase was expressed as millimoles of H_2O_2 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. 0.1mL of the test sample was mixed with 0.5 mL of 10% TCA and 0.5 mL of 75% TBA was added to it. The mixture was then placed in a water bath at 80°C for 45 minutes. The resulting pink solution's absorbance was measured against a reference blank of distilled water at 532 nm. 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[13]. The Lipid peroxidation was expressed as µg/mg protein.

Statistical Analysis: The statistical analysis of data collected from this study was done using the SPSS-8.0 statistical software package[14] for analysis of data. The data was presented as Mean ± Standard Error of Mean (SEM) and statistical analysis was carried out using the student t-test and 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 kidney: The co-treatment group showed mild degenerative changes in the tissues of the kidney; atrophy in the glomeruli evident by widened capsular space. In groups treated with 100 mg/kg of bitter leaf, there were no changes in the histomorphology of the kidney. There was histomorphologic distortion in the proximal and distal convoluted tubules of rats treated with 100 mg/kg of African eggplant as shown in figure 1.

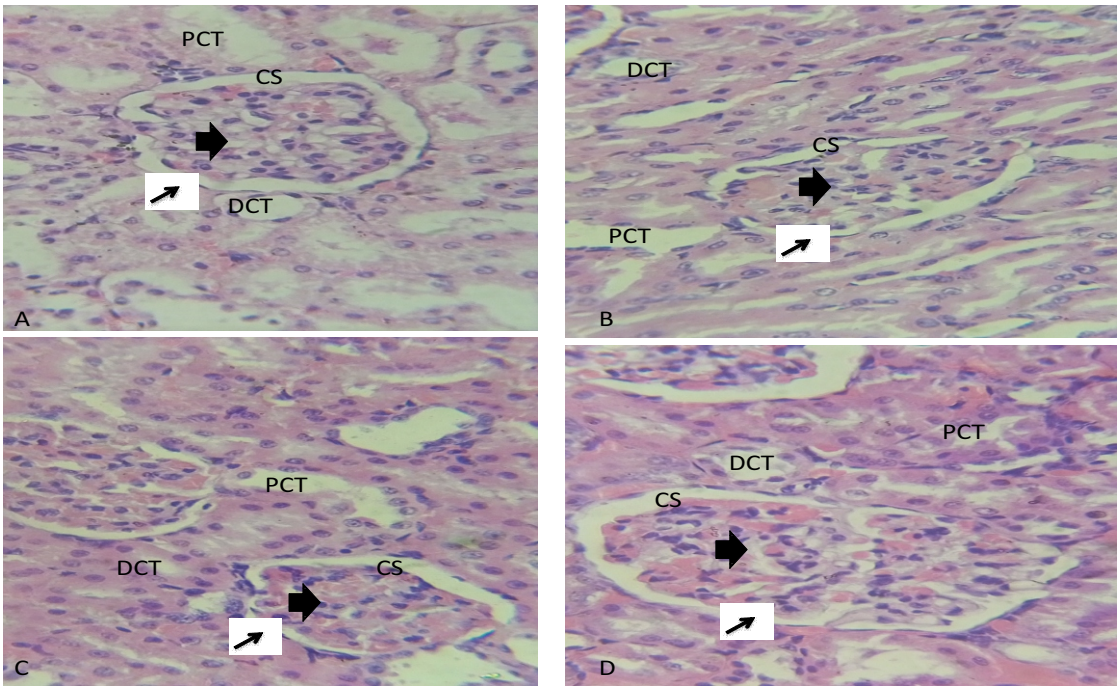
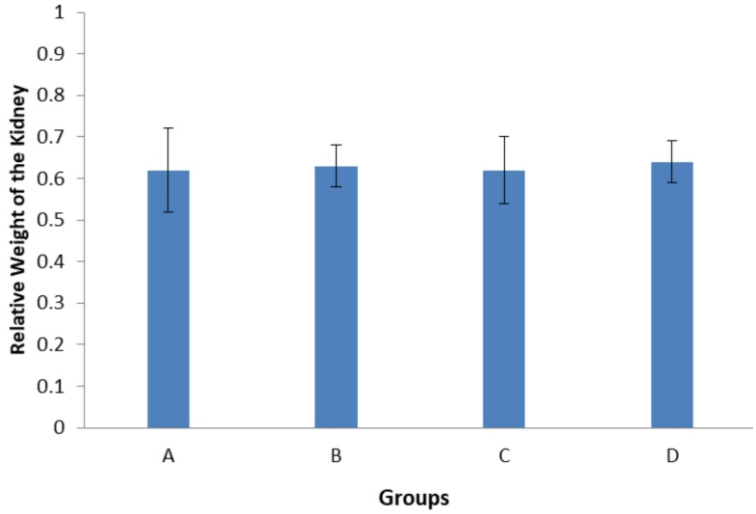


Figure 1a-d: Photomicrograph of kidney tissue following administrations of ethanol extracts of African eggplant only, bitter leaf only and a combination of both. (H/E X400)
Figure 1a (control), distal and proximal convoluted tubules (DCT&PCT), Glomerulus (black thick arrow), simple squamous epithelia cells (black thin arrow) and capsular space (CS), figure 1b (100mg/ kg of African eggplant), epithelial cells (black thin arrow), glomerulus (black thick arrow) and capsular space (CS). Figure 1c (100mg /kg of

bitter leaf plant), capsular space (CS) and glomerulus (black thick arrow), epithelial cells (black thin arrow) and the proximal and distal convoluted tubules (PCT&DCT). Figure 1d (100mg/kg of African eggplant and 100mg/kg of bitter leaf), slight degenerative changes at the glomerulus layer (black thick arrow), proximal and distal convoluted tubules (PCT&DCT).

Effect of the oral administration of the ethanol extract of African eggplant and bitter leaf on the weight of the kidney: As shown in figure 2, only group C rats have the same relative kidney weight when compared with the control group. Other groups showed increase in the relative kidney weight when compared to the control group.



A: control group, B: 100 mg/kg African eggplant, C: 100 mg/kg bitter leaf plant, D: 100 mg/kg of African eggplant and 100 mg/kg of bitter leaf plant

Figure 2: Chart showing relationship between the kidney weights in the different administrations of African eggplant only, bitter leaf only and administration of both.

Effect of the oral administration of the ethanol extract of African eggplant and bitter leaf on the antioxidant enzymes activities in the kidney: When compared to the control group observation from Table 1 shows significant changes ($P < 0.05$) in the results of the administration of ethanol leaf extract of African eggplant and bitter leaf plant on antioxidant enzymes activities in the kidney of male wistar rat. GSH activity across all groups increased, SOD activity in group C rats significantly increased with significant decrease in group D rats. CAT activity across all groups decreased significantly while MDA activity changed significantly only in group B rats, 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 kidney in male Wistar rats

Grps	Treatment	GSH (µmol/ml)	SOD(µmol/ml/min/mg pro)	CAT(µmol/ml/ min/mg pro)	MDA (µmol/ml)
A	Control	14.13±0.11	14.11±3.41	18.34±0.77	3.60±2.11
B	African eggplant	56.21±21.50[a]	11.12±1.73	2.54±0.49 [a]	1.51±0.50[a]
C	Bitter leaf plant	15.69±0.30[a]	18.19±0.47[a]	15.09±0.25 [a]	3.69±0.98
D	Bitter leaf plant and Africa eggplant	62.19±4.78 [a]	2.42±0.75 [a]	11.58±0.64 [a]	4.68±0.35

Each value is an expression of mean ± SEM. ($P < 0.05$)
[a] - Values were significant when compared to group A

DISCUSSION

The toxic effects of traditional medicines depend greatly on the chemical composition of the plant. Even with low-toxicity extracts, traditional herbal treatments might cause toxicity if exposed to for an extended period of time [15]. Several medicinal plants usually have toxic effects on the kidney according to some experimental studies. Antioxidant enzymes are capable of stabilizing or deactivating free radicals before they attack cellular components.

They act by reducing the energy of the free radicals or by giving up some of their electrons for use, thereby causing them to become stable [16]. Many antioxidant enzymes constitute one or more of the defense systems of the body especially in lipid metabolism that produces free radicals or reactive oxygen species (ROS). In preserving health, aging, and age-related disorders, the interplay between the trio of free radicals, antioxidants, and diseases is critical [17]. In biological organisms, the antioxidant molecules that make up the antioxidant defense systems work at varying levels [18].

This present study revealed that the administration of 100 mg/kg of African eggplant caused abnormal changes in GSH and CAT activity in the kidney across all groups. The increase in GSH activity of the kidney indicate the presence of toxic material[19]. The increasing level of GSH in cells indicate oxidative stress and presence of free radicals. The decrease in CAT enzyme activity across all groups indicate the presence of high level of H_2O_2. In the kidney, the main function of CAT and GSH is to break down H_2O_2 to non- toxic oxygen and water. In groups C and D rats, the increase in MDA activity indicate high level of lipid peroxidation as 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 detected is a frequent indicator of oxidative stress and antioxidant status [20], SODs are a family of enzymes that catalyze the dismutation of superoxide radicals (O_2) to molecular oxygen (O_2) and hydrogen peroxide (H_2O_2), offering cellular protection against reactive oxygen species [21]. The decrease in SOD seen in group D rats signify low levels of cellular protection against reactive oxygen species. The increase in the level of GSH is not understood. The decrease in the major antioxidant enzyme of the kidney (SOD and CAT) and increase in lipid peroxidation (MDA) in rats subjected to co-treatment shows the toxicity of eggplant and bitter leaf to kidney and also the cause of atrophic changes in the glomerulus.

The group administered both extracts showed mild degenerative changes in the tissues of the kidney; atrophy in the glomeruli evident by widened capsular space, Odukoya et al.[22] reported progressive kidney tissues degeneration in kidney toxicity, affecting the tubular system, vascular components and the glomeruli consistent with some of the findings in this study in a milder way. According to Maina [23], the change in organ weight could be due to the fact that the plants extract has toxic effects on the kidney which may account for the increase in its size. The kidney is one of the organs involved in detoxification processes in the body and the administration of *Solanum macrocarpon* alongside the bitter leaf treatment may place high level of detoxification demands on the kidneys.

CONCLUSION

This study showed that the administration of bitter leaf and African eggplant may cause increase in lipid peroxidation, decrease in SOD and CAT, and degenerative changes in the tissue of the kidney. Therefore, the combination of these vegetables should be taken with caution.

Declaration of Conflict of interests: All authors wish to declare no conflict of interests

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