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Comparative Study on the Effect of Aqueous Extract of Moringa Oleifera and Vernonia Amygdalina Leaves on Some Biochemical Indices of Male Wistar Rats

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ABSTRACT

Metabolic disturbances are sometimes due to alterations in some biochemical parameters and thereby become an indicator for the assessment of metabolic syndrome. The present study evaluates the comparative effects of aqueous extract of *Moringa oleifera* and *Vernonia amygdalina* on some biochemical indices in male adult Wister rats. Thirty-two adult male Wister rats of average weight of 152 g. Rats were randomly assigned into four groups of eight each. Group one served as the control and each rat in this group received 2ml of distilled water orally and daily. Groups two and three received 2.5 g/ml of *M. oleifera* and *V. amygdalina* per body weight orally and daily. Group four received 2.5g/ml of *M. oleifera* with 2.5g of *V. amygdalina* per body weight orally and daily. Administration of the extract was done for 28 days. All the animals were sacrificed on the 29th day by jugular puncture; part of their blood was collected into plain bottle and spun to obtain serum, and part into EDTA bottle for other analyses. It was observed that a significant increase in total protein was recorded for the group administered *V. amygdalina* and the combination of *M. oleifera* with *V. amygdalina.* A significant increase ($p < 0.05$) in globulin concentration was observed in the group administered only *V. amygdalina* as well as the group administered the combination. *M. oleifera* increased serum albumin compared to *V. amygdalina* and the combination, but decreased the serum bilirubin and creatinine concentration relative to *V. amygdalina* and the combination. A significant increase ($p < 0.05$) in urea level was observed in the group administered the combination of *M. oleifera* and *V. amygdalina* and the group administered only *M. oleifera* when compared to the control value. It is therefore inferred that, aqueous extract of *Moringa oleifera* and *Vernonia amygdalina* has effects on biochemical indices of rats and can be exploited for adjustment purposes when imbalances in these biochemical indices exist and for monitoring some metabolic disturbances.

Key words: Moringa oleifera, Vernonia amygdalina, biochemical indices, metabolic disorder.

INTRODUCTION

Moringa oleifera belongs to the order/family, Moringaceae. It is the most widely cultivated species of a monogeneric family, the moringaceae that is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh

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and Afghanistan. This rapidly-growing tree is also well known as: Horseradish tree, Drumstick tree, Benzolive tree, Kelor, Marango, Mlonge, Moonga, Mulangay, Ben oil tree. It is popularly known in Nigeria by the Igala-Hausa as 'Geligedi', Ibo as 'Ikwe oyibo' and 'Oku ghare ite' Yoruba as 'Ewe ile' and 'Ewe igbale'.

Moringa oleifera trees have been used to combat malnutrition, especially among infants and nursing mothers. It is a natural nutrition for the tropics because the leaves can be eaten fresh, cooked or stored as dried powder for many months without refrigeration, and with nutritional values [2]. *M. oleifera* leaves contain more vitamin A than carrots, more calcium than milk, more iron than spinach, more vitamins than oranges and more potassium than bananas and its protein quality rivals that of milk and eggs [1, 2].

Phytochemicals of Moringa species reveal that the plant family is rich in compounds containing the simple sugar, rhamnose, and it is rich in a fairly unique group of compounds called glucosinolates and isothiocyanates [3]. It has antibiotic activity against a wide range of bacteria and fungi [4] and has shown cancer preventive potential [5].

Vernonia amygdalina is a member of the Asteraceae family. It is a small shrub that grows in the tropical Africa. Typically it grows to a height of 2-5cm. the leaves are elliptical and up to 20cm long with a rough bark [6]. It is popularly known as bitter leaf in English because of its bitter taste. African common names includes; onugbu or olubu (Igbo), ewuro (Yoruba), Ilo (Igala), ityuna (Tiv), grawa (Amharic), etidot (Ibibio), muluuza (Luganda), labwori (Acholi), olusia (Luo), and ndole (Cameroon) [7, 8].

The leaves are dark-green colored with a characteristic odor and a bitter taste. The specie is indigenous to tropical Africa and is found wild or cultivated all over sub-Saharan Africa [9]. The leaves are eaten after crushing and washing thoroughly to remove the bitterness. It has also shown antihelmintic and anti-tumoriagenic properties [10]. Research also confirmed that the leaf extract demonstrated hypoglycemic and hypolipidemic effects in experimental animals [11]. It inhibits growth signals of cancerous cells [12,13], suppression of metastasis of cancerous cells in the body by the inhibition of NFKB, an anti-apoptic transcription factors as demonstrated in animal studies [14], reduction of estrogen level in the body by the suppression of aromatase activity, provision of antioxidant benefits [15], enhancement of the immune system [16], decreased blood glucose by 50% compared to untreated diabetic animals in a study conducted using streptozotocin-induced diabetic laboratory animals [11] and *in vitro* antihelmintic and antiparasitic properties [17].

MATERIALS AND METHODS

Reagents and chemicals

All reagents and chemicals used for these studies were all of analytical grade and were obtained from reputable companies (TECO Diagnostics, 1268 N Lakeview Ave Anaheim, C.A 92807 USA, and BDH (British Drug House), Poole dorset, England).

Preparation of plant used and animal care

Moringa oleifera

Fresh leaves of about 500 grams of *Moringa oleifera* were obtained from Mr. Emmanuel T. Friday's farm of Kogi State University, Anyigba and identified by Professor S.S Usman of the Botanist Department, Kogi State University, Anyigba. It was neat cleaned with clean water and spread over the laboratory bench to air dry at 25 ^{o}C. The dried leaves were ground into powdered form using grinding engine and filtered with sieve of lowest mesh diameter followed by proper aqueous extraction.

Vernonia amygdalina

Fresh leaves of about 500bgrams of *Vernonia amygdalina* were obtained from the Faculty of Agriculture farm, Kogi State University, Anyigba and identified by Professor S.S Usman of the Botany Department, Kogi State

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University, Anyigba. It was also cleaned and air dried in the laboratory at 25 ^{o}C. The dried leaves were ground into powdered form and filtered using sieve of lowest mesh diameter followed by proper aqueous extraction.

Extraction procedure

Extraction procedure was carried out using a modified method of Adedapo *et al* where 50 g of the sieved powdered leaves was soaked in 500 ml of distilled water for 48 hours and was re-sieved. The filtrate obtained was evaporated to dryness at 98-102 ^{0}C using water bath. The concentrate (7 g) was collected, weighed and dissolved in distilled water before administered to the animals in g/kg body weight [26].

Experimental animals/design

Thirty two adult male Wister albino rats were obtained from the Laboratory Unit, Department of Biochemistry, Kogi State University, Anyigba. The rats were acclimatized to the animal house environment for one week before they were randomly assigned to four groups. They were housed in four standard plastic rat cages and were kept under 12 hours light / dark cycle and fed with Growers' marsh with water provided *ad libitum*. Exactly 2 mls of distilled water was given to rats in Group 1 (the control group), 2.5 g/ml of only *Moringa oleifera* and *Vernonia amygdalina* were administered to the animals in group 2 and 3 respectively. Group 4 rats were administered with 2.5g/ml each of both aqueous leaf extract of *Moringa oleifera* combined with *Vernonia amygdalina*, according to their body weights in kilogram. At the end of the treatment, the rats were fasted over the night and later sacrificed by jugular puncture. Exactly 5 ml of the blood was collected from each rat into a free clean anticoagulant sample container and was allowed to stand at ambient temperature until clotting took place and thereafter centrifuged at 1000 rpm for 5 minutes. The serum was carefully removed with the aid of a syringe and transferred into other plain bottles. The collected sera were used for biochemical analysis.

Determination of serum total proteins

The Biuret method was used for this determination. It is based on the principle that at alkaline pH, proteins form a stable violet-colored complex with copper II ions which is spectrophotometrically measured. The depth of the colour obtained is a measure of bonds present in the sample. Reagent composition is; Sodium hydroxide (0.47M), Potassium iodide (23.3mM), Copper II Sulphate (6.5mM), Sodium-Potassium Tartarate (22.1mM) and aqueous protein solution (standard) 5g/dl as preservative. Total protein concentration was determined spectrophotometrically and calculated using the relation = Abs of sample/abs of standard × conc of standard (g/dl). Where Abs = absorbance

Determination of the concentration of total albumin in serum

The Bromocresol green method was used for this assay. This is based on the principle that albumin at a certain pH is specifically combined with bromocresol green to produce a coloured complex which is measured spectrophotometrically.

Reagents composition is; Succinate buffer; pH 4.2 (50mM), Bromocresol green (0.75g/l), Surfactant and albumin standard (5g/dl) was used as preservative. Albumin concentration was calculated using the relation;

Albumin conc. = Abs of sample/Abs of standard × conc. of standard (g/dl)

Estimation of globulin in serum

These were estimated by the differences between the Total protein and albumin of each serum sample using the relation;

Globulin conc. = Total protein conc. – Albumin conc. (g/dl)

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Determination of creatinine in serum

Serum creatinine level was determined using the method described by Jaffe, [17].

Reagents composition is; Picric acid (55 mM), Sodium carbonates (50 mM), NaOH (0.40 M)

Procedure

Test tubes were labeled blank, standard and sample. Aliquots (I ml) of the working reagent were dispensed into each tube. The sample (0.1 ml) and standard (0.1 ml) were pipetted into corresponding tubes. The absorbance were read at 546 nm after 20 seconds and were calculated using;

Abs of sample/Abs of standard X 2 = mg creatinine/dl

Determination of urea in serum

The Berthelot reaction has long been used for the measurement of urea and ammonia. The present method is the modified Berthelot method as described by Tobacco, [18].

Principle

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

$$NH_3 + \text{Salicylate} + \text{Hypochlorite} \longrightarrow \text{2, 2-Dicarboxyindophenol}$$

The Blood Urea Nitrogen (BUN) colorimetric procedure is a modification of the Berthelot reaction. Urea is converted to ammonia by the use of urease. Ammonium ion then reacts with a mixture of salicylate, sodium nitroprusside, and hypochlorite to yield a blue-green chromophore. The intensity of the color formed is proportional to the urea concentration in the sample.

Reagent Composition

When reconstituted as directed, the reagent for BUN contains the following:

After reconstitution with distilled water, BUN enzyme reagent contains: Urease 10, 0000 U/L, Sodium Salicylate 6.0 mmol/L, stabilizers, and a buffer.

BUN Color Developer contains Sodium Hypochlorite, 6 mmol/L and Sodium Hydroxide,

13 mmol/L.

Urea Nitrogen Standard (20 mg/dl) (A stabilizer solution of urea equivalent to 20 mg of Urea Nitrogen/100 ml).

Procedure

BUN Enzyme Reagent (1.5 ml) was dispensed into labeled test tubes and allowed to equilibrate at room temperature. Thereafter 0.01 ml of sample was added to the respective tubes and gently mixed. Deionized water was used as sample for the reagent blank. All tubes were incubated for five minutes at 37 ^{0}C. BUN color developer was then added and mixed gently. This was incubated for 5 minutes at 37 ^{0}C. The spectrophotometer was zeroed with the reagent blank at a wavelength of 630 nm. The absorbance of each tube was read and recorded.

Calculations

(Abs of Sample)/ (Abs of Standard) X Conc. of Standard = Urea Nitrogen (mg/dl).

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Statistical analysis

Results obtained from the study were presented using appropriate tables, charts, graphs or figure. Data obtained was subjected to statistical analysis using SPSS version 16 (F- test and Student's t-test) for the purpose of comparison between the mean study groups and confidence interval was put at 95 % ($p < 0.05$).

RESULTS AND DISCUSION

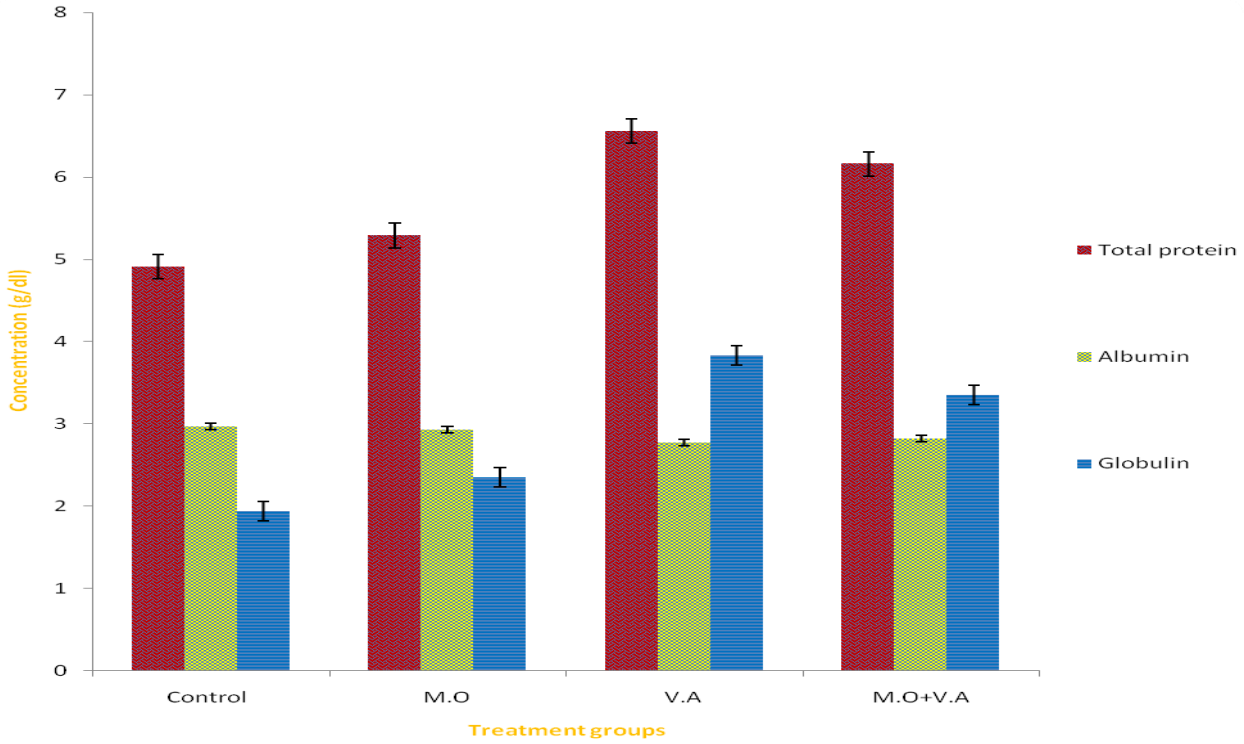

Fig. 1: Serum Total Protein, Albumin and Globulin concentration of rats administered *M. oleifera (M.O)* and *V. amygdalina (V.A)*

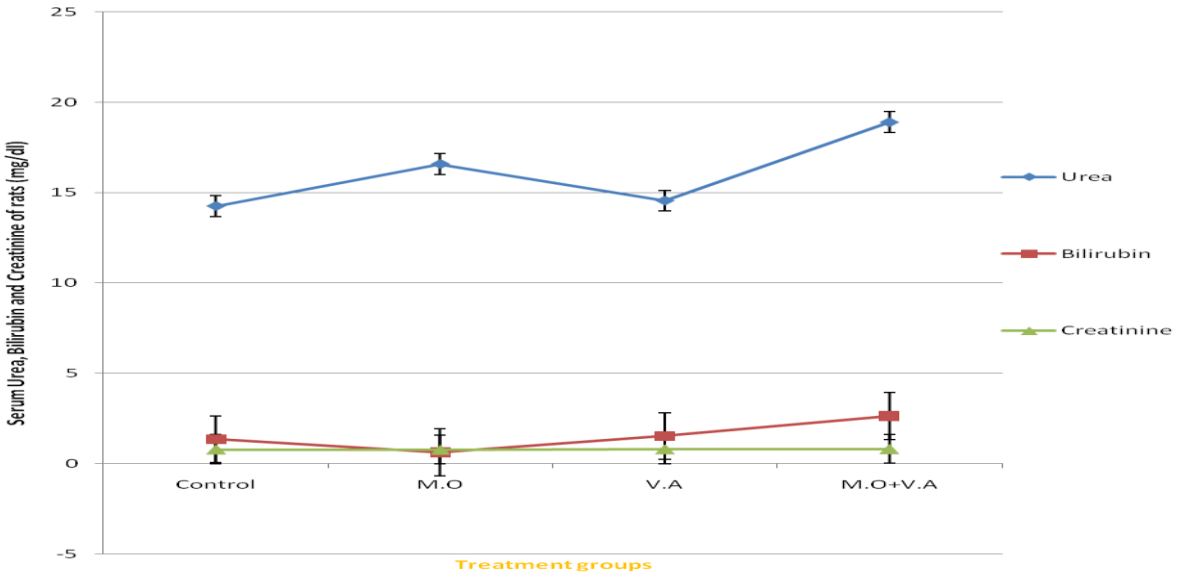

Fig. 2: Serum Urea, Bilirubin and Creatinine concentrations of rats administered *M. oleifera* and *V. amygdalina* extact

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Significant increase ($p < 0.05$) in the serum protein content of the extract of *M. oleifera* and *V. amygdalina* is in agreement with the finding of Fuglie [1], that the protein quality of *M. oleifera* leaves rivals that of milk and eggs, and that it is an excellent source of sulphur-containing amino acids methionine and cysteine. Also fresh leaves of *V. amygdalina* contain protein but at low level in the form of enzymes and may not be an important dietary protein [19]. The non-significant difference ($p > 0.05$) observed in the animals for total proteins could be an indication that, certain toxicants such as isothiocyanate and glycoside cyanides in *V. amygdalina* may cause stress-mediated mobilization of protein to cope with the detrimental condition of administration imposed on the rats [20]. The protein so mobilized is one of the strategies employed to meet the energy required to sustain increased physical activity, biotransformation and excretion of the toxicants. It was reported by [21] that an appreciable high amount of iron is present in *V. amygdalina* which is required for hemoglobin formation.

The observable significant increase ($p < 0.05$) in globulin level recorded after treatment with both plant leaf extract support the antimicrobial action of these plants since globulins are principally responsible for both the natural and acquired immunity that an individual has against invading organisms [22].

Non-significant reduction ($p > 0.05$) in albumin level for all the extracts may be due primarily to reduction in synthesis by the liver and secondarily, to reduction in protein level [23, 24]. Albumin apart from being a useful indicator of the integrity of the glomerular membrane is also important in determining severity of disease when present [25].

A decrease level ($p < 0.05$) in bilirubin level may be due to the fact that the extract of the plant contains active ingredients that prevent the on-set of hemolytic anemia. Bilirubin is a breakdown product of the haeme component of the hemoglobin molecule. Total serum bilirubin is elevated in animals with hemolytic anemia which could be as a result indirect-reacting bilirubin. The degree to which bilirubin is elevated in hemolytic anemia is a function of the rate of red cell destruction and the capacity of the liver to excrete the newly formed bilirubin [26].

An increase level ($p < 0.05$) in urea level of the rats administered these plant extracts could be as a result of high protein diet, intestinal hemorrhage, dehydration, severe hemorrhage, shock, etc. urea level could be decreased due to; liver failure, low protein diet, anabolic steroids, diabetes insipidus [27]. Urea is one of a number of non-protein nitrogenous substances that accumulate in the plasma when renal excretion is reduced. This follows the same trend with serum creatinine of the rats.

In conclusion, our findings revealed that, *M. oleifera* and *V. amygdalina* increased serum urea level, serum globulin and protein but reduce bilirubin level. Therefore, the administration of the plant's extract singly or synergistically can be a source of therapy for the management of metabolic disorders.

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