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Biochemical Effects Of Ethanol Leaf Extract Of A Postpartum Medicinal Plant, *Combretum Racemosum* On Lactating Female Abino Rats

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Abstract
This study evaluated the effect of ethanol leaf extract of a postpartum medicinal plant, Combretum racemosum on some biochemical parameters on lactating female albino rats. The experimental animals (20 lactating female Rats and 5 non-lactating female rats) of weight between 130g – 184g were grouped into five groups. Group 1 (non-lactating female rats) were fed with normal Rat feed (normal control), Group 2 (lactating rats) fed with normal rats feed, group 3 (lactating rats) fed with normal rat feed +1000mg/kg of the extract, Group 4 (lactating rats) fed with normal Rat feed +1500mg/kg of the extract, group 5 (lactating rats) fed with normal rats feed + 2000mg/kg of the extract. After 14days of oral administration of the extract, the rats were sacrificed, blood, liver and kidney samples were collected for analysis. Antioxidant activities, liver and kidney function parameters were determined. Data obtained were analyzed by inferential Statistic. Result showed that percentage weight gain of the experimental animals significantly decreased as the dose increases down the group compared to the control groups. Total protein, albumin and globulin of the test groups showed a significant increase in concentration compared to negative control group while liver enzymes, , aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) concentration significantly decreased in test groups compared to the normal control groups. Result of renal function parameters indicated a significant decrease in urea, creatinine and chloride compared with the normal control group. Result of the antioxidant effect of the extract showed a significance decrease in Glutathione peroxidase (GPx), Catalase (CAT), and Superoxide dismutase (SOD) concentration of negative control group compared to test groups. Conclusively, this study demonstrated the antioxidant, renocellular and hepatoprotective potential of ethanol leaf extract of Combretum racemosum on postpartum lactating female albino rats.

Keywords; *Combretum racemosum, Renocellular, Hepatoprotective potential, antioxidant activity.*

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I. Introduction

The use of plant extracts in parturition in Nigeria and other the developing countries of the world cannot be over emphasized. Herbal medicine is a reality of many cultures especially in Africa particularly in Nigeria (Orabueze *et al.,* 2017; Madara *et al.* 2018) such that despite the advancement of orthodox medicine it is still well entrenched and practiced because of better cultural acceptability and better compatibility.

Combretum racemoskum is a vital component of Aju Mbaise polyherbal extract used in South East Nigeria by many women to enhance labour, remove retained placenta after delivery and for managing pains from post-natal and menstrual cramps and as well promote endometrial contraction when administered to women after child birth. (Nnadiukwu and Lawrence, 2020)

Combretum racemosum is locally known as Ebiodo among the Urhobos in Nigeria, Ogbenejobo *et al* (2014), Uboli among Mbaise community and very popular as it is one of the major herbs of Aju Mbaise polyherbal extract. The plant has been used for several years in African traditional medical practices and as a condiment in soup, in addition to its anthelmintic (Okonosa *et al* 2006), Trypanocidal and antimicrobial properties for genito-urinary and gastrointestinal infections (Onucha, *et al* 2005), The plant is also used for the treatment of hemorrhoids, convulsion, coughing, tuberculosis, toothache, stomach pains, abdominal disorder, fever and male sterility. (Eleganmi *et al* 2007) (Eloff, Katereme, Mcgaw 2018)

The leaf *of Combretum racemosum* is used in Sierra Leone to season soup (Daziel, 2020**).** The young leaf is said to be anthelmintic (Daziel, 2020). It is used in Gambia to reduce the activity of roundworms in children. The leafy twigs are the basis for various preparation used in Casamance of Senegal against internal parasites. The leaf-sap when mixed with water produces a greenish liquid which gels in time and the jelly is taken in Ivory Coast for male sterility (Bouquet and Debray**,**2017). The bark produces a gum which is reported to be used in Gambia for toothache.

The leaves are used in traditional medicines by inhabitants of the eastern parts of Nigeria for treatment of ulcers, diarrhea, and menorrhagia. It has also been documented as a remedy for treatment of some parasitic, bacteria, and fungal infections and has a folkloric reputation as trypanocidal, antihelminthics and antimicrobial agents and also claimed to be effective against stomach pains, dysentery, abdominal disorder and fever. (Onucha *et al* 2020).

II. Methodologies

Preparation Of Plant Extracts (Sample)

Plant extract was prepared in accordance with the method used by Orieke *et al.,* (2019). Freshly collected leaves of *Combretum racemosum* were properly washed and air dried under a shade for about 7 days and were thereafter pulverized into fine powdered, using a locally fabricated grinding machine. Ethanol extraction was carried out for about 48 hours at room temperature with intermittent shaking while the resultant mixture was filtered using dry white clean cloth and the filtrate was allowed to evaporated at 40^{0c} temperature. The sample was stored in a refrigerator until ready for use.

Animals

A total of 45 adult rats (30 female and 15 male) was obtained from the laboratory animal production unit of the Department Biochemistry, Abia State University, Uturu for the study. The animals were housed in aluminum cages, under standard conditions 12/12h light-dark cycle at temperature of 25±2^{o}C and were fed with standard grower pellets (Grand Cereals Ltd, Abia State) and had access to clean drinking water for fourteen days for acclimatization before the initiation of experiment.

Experimental Design

The study was carried out in two stages; the first being the process of ensuring that the animals become pregnant for the experimental purposes and it involved grouping the animals into 15 groups of two female rat and one male rat for a period of 28 days for breeding and gestation. In the second stage20 lactating female rats and 5 normal female rats were isolated put in different cages for the second stage of the experiment for a period of 18 days. The design was carried using 20 lactating female rats and 5 non – lactating rats assigned to 5 groups of 5 rats each and treated as follows:

Treatment protocol for the experimental rats

Group	No of rats	Treatment
1	**5**	Non-lactating Female Rat (Normal Control)
2	5	Lactating female rats (Negative control)
3	5	Lactating female rats + 1000mg/kg of extract
4	5	Lactating female rats + 1500mg/kg of extract
5	5	Lactating female+ 200j0mg/kg of the extract.

The oral administration of the extract to rats lasted for 18 days. The rats were sacrificed thereafter and blood samples were collected into plain bottles for biochemical analysis. The Liver and kidney tissue of the animals were also collected into plain sample bottles containing 10% formaldehyde solution which were used for preparation of tissue homogenates for histopathological studies.

Determination Of The Body Weight Of The Animal Before And After The Experiment Period.

The initial bodyweight (W1) of the experimental rats (Weight before giving the extract) and the final body weight (W2) of the experimental rats (weight before the rats were sacrificed) were taken using a weighing balance and weights recorded. The weight gain (W3) was calculated by subtracting W2 from W1.
The percentage weight gain was calculated using the formular; % = W3/W1 x 100/1

Determination Of Liver Function Parameters

All liver function parameters were determined using the respective commercial test kits and following protocols for the parameters as reported by Ugbogu *et al*., (2017).

Alanine Aminotransferase (ALT)

Kit: RANDOX

Reagent: R1 (A combination of 100 mmol/L of Phosphate buffer at P^{H} 7.4,200 mmol/L of L-alanine and 2 mmol/L of α-oxoglutarate). R2 (2,4-dinitrophenylhydrazine) and 5% NaOH **solution.**

Principle:

SGPT (ALT) catalyses the transfer of amino group from L-alanine to α-ketoglutarate to yield pyruvate and L-glutamate. Lactate dehydrogenase then converts pyruvate and NADH into lactate and NAD^+. The conversion of NADH to NAD^+ decreases the absorption at 340nm. The rate of decease in absorbance is measured at 546nm and is proportional to the SGPT (ALT) activity.

L-alanine +α- ketoglutarate $\xrightarrow{AST}$ L-glutamate + pyruvate

Pyruvate + NADH + H $\xrightarrow{+LDH}$ Lactate + NAD^+.

Elevation of SGPT (ALT) activity is found in liver and kidney disease such as infectious or toxic hepatitis, infectious mononucleosis and cirrhosis. Moderate increase is also found in obstructive jaundice, metastasis carcinoma, hepatic congestion and myocardial infarction.

SGPT levels may be decreased in patients undergoing long term haemodialysis without supplemental vitamin therapy. The normal ranged values in the serum are 0-12 U/L.

Procedure: Two test tubes were set up in a test tube rack and labeled "Blank: and test. 0.1ml of the sample (test serum) was introduced into the test tube while 0.1ml of distilled water was pipetted into the blank test tube. 0.5ml of ALT reagent R_1 was then be pipette into each of the tubes and mixed. The mixture was incubated for 30 minutes at 37^{0}C before the addition of 0.5ml of reagent R_2 to each of the tubes and also incubated at 20-25^{0}C for 20 minutes. 5ml of Sodium Hydroxide was then added to each tube and allowed to stand for 5 minutes before absorbance was read against the reagent blank on a spectrophotometer (722 N, CHINA) at 546nm. ALT activity in the serum was then obtained by tracing the equivalent absorbance of the sample on the ALT absorbance chart and finding its corresponding ALT activity value in U/L.

Aspartate aminotransferase (AST)

Kit: RANDOX

Reagent: R1 (A combination of 100 mmol/L of Phosphate buffer at P^H 7.4,100 mmol/L of L-aspartate and 2 mmol/L of α-oxoglutarate). R2 (2,4-dinitrophenylhydrazine) and 5% NaOH solution.

Principle: PRINCIPLE

AST catalyzes the transfer of the amino group from L aspartate to α-ketoglutarate to yield oxaloacetate and L-glutamate. Malate dehydrogenase (MDH), then converts oxaloacetate and NADH to malate and NAD^+. The conversion of NADH to NAD^+ decreases the absorbance at 340nm, the rate of which is proportional to the AST activity.

AST

L-aspartate +α- ketoglutarate $\xrightarrow{AST}$ Oxaloacetate + L-glutamate

Oxaloacetate + NADH + H^{+MDH} $\longrightarrow$ Malate +NAD^+.

Organ rich in AST are heart, liver and skeletal muscle. When any of these organs are damaged, the serum AST level rises in proportion to the severity of damage. In myocardial infarction AST starts increasing by 3 – 9 hours, peaks on second day return to normal on 4^{th} – 6^{th} day. In hepatitis, AST peaks usually between 7 – 12 days and any increase up to 100 times. Increased levels AST are also found in mononucleosis, pancreatitis and trauma of skeletal muscle, renal necrosis and cerebral necrosis. The normal ranged values in the serum are 0 – 12 U/L.

Procedure: A blank test tube was set up into which 0.1ml of distilled water was be pipetted. 0.1ml of the test serum was also pipetted into a second test tube labelled "test". AST reagentR_1 (0.5ml) was added to each test tube, mixed and incubated at 37^{0}C for 30 minutes. After the incubation, 0.5ml of AST reagentR_2 was added to each test tube and allowed to stand for 20 minutes at 20-25^{0}C before 5.0ml of 0.4mol/ liter Sodium Hydroxide was added to each allowed to stand for 5 minutes before reading absorbance against the reagent blank on a spectrophotometer (722N, CHINA) at wavelength 546nm. AST activity in the serum was then obtained by finding an equivalent values of absorbance on the standard chart and obtaining its corresponding activity value in U/L.

Alkaline phosphatase (ALP) activity

Kit: RANDOX

Reagents: R1a (A combination of 1 mol/L of Diethanolamine buffer at pH 9.8 and 0.5 mmol/L of $MgCl_2$). R1b (10 mmol/L of p-nitrophenylphosphate).

Principle: The substrate, p-nitrophenyl phosphate (PNPP) is hydrolyzed by ALP to p-nitrophenol and phosphoric acid. Some divalent ions like Mg^{++} are added to the system which acts as activators. PNPP is

colorless in acid or alkaline medium while PNP is yellow in colour in the alkaline medium and colorless in the acid medium.

p- nitrophenyl phosphate + H_2O ⟶ p- nitrophenol + H_3PO_4

pNPP + H_20 ⟶ pNP + H_3P0_4

Colorless ⟶ Yellow

Procedure: Three test tubes were set up and labeled test, control and standard respectively into which 0.5ml of Alkaline phosphatase substrate was added. 0.05ml (50µl) of the standard, control and sample (test serum) were added to the corresponding test tubes while distilled water was used for the control. The test tubes were incubated for 10 minutes at 37^{0}C before adding 2.5ml of Alkaline Phosphate color developed at timed intervals. The mixtures were properly mixed before reading absorbance on a spectrophotometer at 590nm after zeroing with the reagent blank.

ALP value in IU/Liter was calculated using the formular

$$\frac{\text{Absorbance of Sample}}{\text{Absorbance of standard D}} \times 50$$

Where 50 is the standard ALP value

Albumin Determination

Method of Doumas *et al.* (1971) using Randox test kits was used for determination of albumin

Principle

The measurement of serum albumin is based on its quantitative binding to the indicator 3,3',5,5'-tetrabromo-m cresol sulphonephthalein (bromocresol green, BCG).

Procedure

Three test tubes were labeled Blank, Standard and Sample. Distilled water (0.01 ml), Standard (0.01 ml) and plasma (0.01 ml) were measured into the three test tubes respectively, then 3.00 ml BCG reagent added to each of the test tubes. The solutions were mixed and incubate for 5 min at 20-25 ^{o}C, then their absorbance was read on a spectrophotometer at 578 nm. After 2 min, the standard's absorbance as well as that of the sample were read and recorded

Concentration of albumin in sample was calculated as:

Serum bilirubin determination

Kit: RANDOX

Reagents: R1 (29 mmol/L Sulphanilic acid and 0.17N Hydrochloric acid). R2 (38.5 mmol/L of Sodium Nitrite). R3 (0.26 mol/L of Caffein and 0.52 mol/L of Sodium benzoate). R4 (0.93 mol/L of Tartrate and 1.9N NaOH).

Principle: Direct (conjugated) bilirubin reacts with diazotized sulphanilic acid in alkaline medium to form a blue coloured complex. Total bilirubin is determined in the presence of caffeine, which releases albumin bound bilirubin by the reaction with diazotized sulphanilic acid.

Procedure: Two test tubes were set up and labeled blank and sample. To the sample test tube, 200 μl of Reagent 1, 50 μl of Reagent 2 and 1000nl of Reagent 3 were added and mixed properly while only 200ml of Reagent 1 and 1000nl of reagent R_3 were added to the blank test tube. The 2 test tubes were then be incubated at 20-25^{0}C for 10 minutes, after which 100 μl of reagent 4 was added to both test tubes. The test tubes were incubated for a further 30 minutes at 25^{0}C before reading absorbance on a spectrophotometer at 560nm after zeroing with blank. To obtain total bilirubin in mg/dl the formular below was used.

Total Bilurubin =10.8 x Absorbance of Sample

Serum total protein determination

Kit: RANDOX

Reagent: R1(A mixture of 100mmol/L of NaOH, 16mmol/L, Na-K-tartrate, 15 mmol/Lof Potassium iodide and 6 mmol/L of CuSO4).

Principle: Cupric ions, in an alkaline medium, interact with protein peptide bonds resulting in the formation of a purple colouredcomplex. The intensity of the colour is proportional to the protein concentration in the sample.

Procedure: Three test tubes were set up labeled test, blank and standard. 1.0ml of the total protein reagent was introduced into each of the test tubes. 20µl of the test sample was collected and introduced into the test tube labeled test. The same volume of the standard reagent was introduced into the test tube labeled standard while

the same volume of distilled water was introduced into the blank test tube. The mixtures were incubated at 20-25^{0}C for 30 minutes before absorbance of both the standard and test were read on a spectrophotometer after zeroing with the blank at 560nm. Total protein content of sample was obtained using the formula below.

Total protein = $\frac{\text{Absorbance of test}}{\text{Absorbance of standard}}$ x Concentration of standard

Where concentration of standard = 5.95mg/dl

DETERMINATION OF RENAL FUNCTION PARAMETERS

All renal function parameters were determined using the respective commercial test kits and following protocols for the parameters as reported by Ugbogu *et al*., (2017).

Urea

Kit: RANDOX

Reagent: R1 (a combination of 116 mmol/L of Sodium nitroprusside and 6 mmol/L of Urease). R2 (120 mmol/L of dilute phenol). R3 (27 mmol/L of dilute Sodium hypochlorite) and 0.14N NaOH.

Principle: This test is based on the fact that urea in serum is hydrolyzed to ammonia in the presence of urease. Hence the amount of ammonia formed is proportional to urea concentration.

Procedure: The contents of vial R1a were transferred into bottle R1b and mix gently forming reagent R1. Also, the contents of bottle R2 was diluted with 660 ml of distilled water and mix thoroughly. Also, the contents of bottle R3 was diluted with 750 ml of distilled water. The test was then ready to start. Three test tubes labelled test, blank and standard were set up. Approximately 100 µl of R1 was pipetted into each of the test tubes and 10µl of the test sample added into the test tube labelled test. The same volume of the standard reagent and distilled water were added to the test tubes labelled standard and blank respectively. The mixtures in the test tubes were incubated at 37^{0}C for 10 minutes and thereafter 2.5 ml of reagent R2 and the same volume of R3 added to each test tube. The contents of each tube were mixed immediately and incubated at 37^{0}C for 15 minutes. At the end, the absorbance of the test and that of the sample were read after zeroing with blank. The urea concentration in the sample was calculated thus:

Urea concentration in mg/dl = $\frac{\text{Absorbance of test}}{\text{Absorbance of standard}}$ x concentration of standard

Where concentration of standard = 78.73 mg/dl

Creatinine

Kit: RANDOX

Reagent: R1a (35 mmol/L of picric acid). R1b (0.32 mol/L of NaOH solution)

Principle: This test is based on the principle that creatinine in alkaline solution reacts with picric acid to form a colored complex. Creatinine concentration is therefore proportional to the amount of colored complex formed.

Procedure: Equal volumes of R1a and R1b were mixed to form the working reagent and then 2 test tubes labeled test and standard were set up. Exactly 2 ml of the working reagent was transferred into each test tube and 0.2 ml (200µl) of the sample added into the test tube labeled test and the same volume of the standard reagent into the standard. The content of each test tube was mixed and after 30 seconds absorbance A_1 for both the standard and test were read and in another 2 minutes' time, absorbance A_2 also for standard and test were read.

Absorbance of test or sample = $A_2 - A_1$

Absorbance of standard = $A_2 - A_1$

Creatinine concentration in mg/dl = $\frac{\text{Absorbance of test}}{\text{Absorbance of standard}}$ x concentration of standard

Where concentration of standard = 2.05 mg/dl

Sodium

Kit: TECO

Reagent: 1. Filtrate reagent (comprising of 2.1 mmol/L of Uranyl acetate 20 mmol/L of Magnesium acetate in ethyl alcohol). 2. Acid reagent (dilute acetic acid). 3. Sodium colour reagent (Potassium ferrocyanide, non-reactive stabilizers and fillers). 4. Sodium standard (150 mEq/L of NaCl solution).

Principle: This test is based on the principle of precipitating sodium, as a triple salt, sodium magnesium uranyl acetate, with the excess uranium being reacted with ferrocyanide to produce a chromophore whose absorbance is inversely proportional with the concentration of sodium in the sample being tested.
Procedure: Three test tubes properly labelled test, standard and blank were set up and 1 ml of filtrate reagent was pipetted into each test tube. Exactly 50µl of the sample was added to the test tube labelled test and the same volume of the standard reagent to the standard and also the same volume of distilled water to the blank. All test tubes were shaken vigorously to ensure adequate mixing of their contents. Each test tube was centrifuged at high speed for 10 minutes to obtain a supernatant above and precipitate below. Care was taken not to disturb the protein precipitate. The test proceeded to the stage with the supernatant only.

Another set of three test tubes still labelled test, standard and blank were set up and 1 ml of the acid reagent was pipetted into each of the tubes. Then 50µl of the supernatants was added to their corresponding test tubes and mixed properly. Also, 50µl of the colour reagent was added to all the tubes and mixed. The spectrophotometer was zeroed with distilled water and then the absorbance of the content of each test tube was read and recorded. The concentration of sodium in the sample was calculated using the expression:

$$\text{Sodium conc. in mEq/L} = \frac{\text{Abs. of blank} - \text{Abs. of test}}{\text{Abs. of blank} - \text{Abs. of standard}} \times \text{Concentration of standard}$$

Where concentration of standard = 150 mEq/L

Chloride
Kit: TECO
Reagent: 1. Chloride reagent (comprising of 0.058 mmol/L of Mercuric Nitrate, 1.75 mmol/L of Mercuric Thiocyanate, 0.74 mmol/L of Mercuric Chloride and 22.3 mmol/L of Ferric Nitrate) with non-reactive ingredients and stabilizers in dilute acid and methanol. 2. Chloride standard (100 mEq/L of NaCl solution).
Principle: Chloride ions form a soluble non-ionized compound with mercuric ions and will displace thiocyanate ions from non-ionized mercuric thiocyanate. The released thiocyanate ions then react with ferric ions to form a color complex whose intensity is proportional to the concentration of chloride in the sample.
Procedure: Three test tubes properly labeled test, standard and blank were set up and 1.5 ml of the chloride reagent was pipetted into each tube. Then 10µl of the sample was added to the test tube labeled test and the same volume of the standard reagent to the standard and also the same volume of distilled water to the blank. All test tubes were shaken to mix and incubated at 25^{0}C for 5 minutes. Then the absorbance of the content of each test tube was read at wavelength 500 nm after zeroing with the blank.
Calculate chloride concentration in mEq/L with the expression:

$$\text{Chloride concentration in mEq/L} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{concentration of standard}$$

Where concentration of standard = 100 mEq/L

Potassium
Kit: TECO
Reagent: 1. Potassium reagent (comprising of 2.1 mmol/L of Sodium Tetraphenylboron, preservatives and thickening agents). 2. Potassium standard (4 mEq/L).
Principle: Sodium tetraphenyl boron in a specifically prepared mixture can produce a colloidal suspension. The turbidity of this suspension is directly proportional to the concentration of potassium in the sample.
Procedure: Three test tubes properly labeled test, standard and blank were set up and 1ml of the chloride reagent was pipette into each tube. Then 10µl of the sample was added to the test tube labelled test and the same volume of the standard reagent to the standard and also the same volume of distilled water to the blank. All test tubes shaken to mix and allow to stand at 25^{0}C for 3 minutes. Then the absorbance of the content of each test tube was read at wavelength 500 nm after zeroing with the blank.
Calculate potassium concentration in mEq/L with the expression:

$$\text{Potassium conc. in mEq/L} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{concentration of standard}$$

Where concentration of standard = 4 mEq/L

Bicarbonate
Kit: TECO
Reagent 1: Carbon dioxide reagent (comprising of 1.8 mmol/L of Phosphoenol pyruvate, 10 mmol/L of Magnesium sulphate, 0.40 mmol/L of Nicotinamideadeninie dinucleotide, 1250 u/l of Malate dehydrogenase,

200 u/l of Phosphoenol pyruvate carboxylase, 2.5 mmol/L of Sodium Oxalates, Buffer solution of P^H 7.0, non-reactive fillers and stabilizers with 0.1 % sodium azide as preservative. 2. Bicarbonate standard which contains 30 mm0l/L of Sodium bicarbonate in an aqueous solution.

Principle: Phoesphoenol pyruvate carboxylase Catalyses the reaction between phosphoenol pyruvate and carbon dioxide (bicarbonate) to form oxaloacetate and phosphate ion. Oxalacetate is reduced to malate with simultaneous oxidation of equimolar amount of reduced nicotinamide adenine dinucleotide (NADH) to NAD; the reaction is catalyzed by malate dehydrogenase. This results in a decrease in absorbance at 340 nm that is directly proportional to the CO2 concentration in the sample.

Procedure: Carbon dioxide (CO_2) reagent was reconstituted with the volume of CO_2 free water indicated on the vial label, mixed by gentle inversion 5-6 times. Three cuvettes labelled test, standard and blank were set up and 1 ml of the reagent was pipetted into each tube and all tubes were incubated for 3 minutes at 37^0C. Exactly 10µl of the sample were added to the cuvette labelled test and the same volume of the standard reagent and distilled water to the standard and blank respectively. The absorbance was read at 340 nm and the bicarbonate concentration calculated thus:

Bicarbonate conc. in mmol/L = $\frac{\text{Abs of blank – Abs of test}}{\text{Abs of blank – Abs of standard}}$ x Conc. of standard

Where concentration of standard = 30 mmol/L

Determination of enzymatic antioxidants on liver tissue homogenates

Estimation of Superoxide dismutase (SOD)

Superoxide dismutase activity was assayed by the method of Arthur and Boyne (1985).

Principle: The method employs xanthine and xanthine oxidase to generate superoxide radicals which reacts with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (I.N.T) to form a red formazan dye. The superoxide dismutase activity is then measured by the degree of inhibition of this reaction. One unit of SOD causes a 50% inhibition rate in the reduction of (I.N.T) under the conditions of the assay.

Procedure: To 0.05ml diluted sample in a test tube was added 1.7ml mixed substrate solution and mixed xanthine oxidase (0.25ml) was added and the initial absorbance taken after 30 seconds. The final absorbance was taken after 3 minutes and units of SOD per gram hemoglobin were extrapolated from a standard curve.

Estimation of catalase

The activity of catalase was assayed by the method of Sinha (1972).

Principle: Dichromate in acetic acid was reduced to chromic acetates, when heated in presence of hydrogen peroxide with the formation of per chronic acid as unstable intermediate. The chronic acetate formed was measured at 570nm. Catalase was allowed to split H_2O_2 for different periods of time. The reaction was stopped at different time intervals by the addition of dichromate acetic acid mixture in 1:3 and the remaining H_2O_2 was determined by measuring chromic acetate calorimetrically.

Procedure: To 0.9ml of distilled water and 0.1ml of plasma in a test tube was added 2ml of H_2O_2 and 2ml phosphate buffer. The reaction was initiated by adding 2ml of dichromate acetic acid reagent to 1ml portion to this mixture. Absorbance of the reaction was taken in 30 seconds interval for 2 minutes. The activity of catalase was expressed as U/ml of plasma (U-micro-moles of H_2O_2 utilized per second.

Estimation of glutathione peroxidase

This was done according to the method of Paglia and Valentine (1967).

Principle: Glutathione (GPx) catalysis the oxidation of reduced glutathione (GSH) by cumene hydroperoxide. In the presence of glutathione reductase (GR) and NADPH, the oxidized glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH and $NADP^+$. The decrease in absorbance at 340nm is measured.

$2GSH + ROOH \longrightarrow GSSG + ROH + H_2O$

$GSSG + NADPH + H^+ \longrightarrow NADP^+ + 2GSH$

Procedure: A known volume, 0.05ml of heparinized whole blood was diluted with 2ml of diluting reagent and this was used for the assay. A volume, 50ml of dilute sample was mixed with 1ml of phosphate buffer + EDTA, glutathione + glutathione reduces + NADPH and cumene hydroperoxide respectively. The initial absorbance of both blank and test were read after per minute and the timer started simultaneously. The absorbances were read again after 1- and 2-minutes intervals at 340nm. The glutathione peroxidase activity was calculated as follows:

U/L of hemolysates = 8H [illegible] DA 340nm / minute.

Statistical analysis

Data generated from the study were analyzed using the Statistical Package for the Social Science (SPSS) version 22.0. Group comparisons were done using the analysis of variance (ANOVA) test. Significant differences between control and experimental were assessed by the least significant difference (LSD). All data were expressed as mean ± standard deviation. P-values less than 0.05 were considered to be statistically significant.

III. Result

Effects of Ethanolic leaf extract of *Combretum racemosum* on body weight changes of postpartum lactating female Albino Rats.

Groups	Treatments	Initial body weight (g)	Final body weight (g)	Weight gain (g)	Percentage weight gain
1	Normal rat Feed (Positive Control)	130.00±5.77^{a}	160.00±2.89^{a}	30.00±2.89^{d}	23.37±3.27^{c}
2	Lactating + rat Feed (Negative Control)	150.67±6.36^{a}	184.33±5.81^{b}	33.67±0.67^{d}	22.46±1.36^{c}
3	Lactating rats+ 1000mg/kg of extract	149.33±5.81^{a}	167.67±5.36^{a}	18.33±0.88^{c}	12.34±0.98^{b}
4	Lactating rats+ 1500mg/kg of extract	149.67±6.12^{a}	160.33±2.91^{a}	10.67±3.38^{b}	7.32±2.48^{ab}
5	Lactating rats+ 2000mg/kg of extract	150.67±7.86^{a}	157.00±6.56^{a}	6.33±1.45^{a}	4.32±1.17^{a}

Value is mean ± standard deviation at triplicate determination (P<0.05). n=5.

Table 3.2 Effects of Ethanolic leaf extract of *Combretum racemosum* on liver function parameters of lactating female Albino Rats.

Treatments	Normal rat Feed (Normal Control)	Lactating rats (Negative Control)	Lactating rats + 1000mg/kg of extract	Lactating rats + 1500mg/kg of extract	Lactating rats + 2000mg/kg of extract
Total protein (g/dl)	8.29±0.10^{b}	4.65±0.27^{a}	8.06±0.14^{b}	7.92±0.03^{b}	8.07±0.08^{b}
Albumin (g/dl)	4.84±0.16^{c}	2.22±0.12^{a}	4.27±0.14^{b}	4.24±0.05^{b}	4.16±0.04^{b}
Globulin (g/dl)	3.45±0.11^{b}	2.43±0.17^{a}	3.78±0.06^{c}	3.68±0.02^{bc}	3.91±0.04^{c}
AST (u/l)	41.67±1.67^{a}	93.00±1.73^{c}	60.33±1.45^{b}	59.33±3.18^{b}	57.33±3.71^{b}
ALT (u/l)	32.67±1.76^{a}	53.33±1.45^{c}	39.67±1.45^{b}	44.33±1.76^{b}	40.33±0.88^{b}
ALP (u/l)	70.67±1.20^{a}	115.67±2.19^{c}	78.67±3.53^{ab}	77.00±1.00^{ab}	80.67±3.84^{b}
Total Bilirubin (mg/dl)	0.45±0.03^{a}	1.26±0.04^{c}	0.50±0.03^{a}	0.64±0.03^{b}	0.64±0.07^{b}

Value is mean ± standard deviation at triplicate determination. (P<0.05). n=5

Table 3.3 Effects of Ethanolic leaf extract of *Combretum racemosum* on renal function parameters of lactating female Albino Rats.

Treatments	Normal rat Feed (normal Control)	Lactating rats (Negative Control)	Lactating rats + 1000mg/kg of extract	Lactating rats + 1500mg/kg of extract	Lactating rats + 2000mg/kg of extract
Urea (mg/dl)	20.67±0.54^{a}	32.00±3.13^{b}	22.90±1.13^{a}	22.90±1.03^{a}	21.90±0.66^{a}
Creatinine (mg/dl)	0.62±0.02^{a}	1.97±0.05^{c}	0.94±0.04^{b}	1.04±0.07^{b}	0.90±0.03^{b}
Na^{+}(mEq/L)	129.23±0.96^{a}	132.97±1.04^{b}	131.40±0.61^{ab}	131.17±0.64^{ab}	131.10±0.75^{ab}
K^{+} (mEq/L)	4.48±0.08^{b}	4.14±0.09^{a}	4.30±0.05^{ab}	4.47±0.06^{b}	4.35±0.14^{ab}
Cl^{-} (mEq/L)	88.87±0.80^{a}	96.60±1.86^{b}	91.57±1.17^{a}	91.57±0.89^{a}	90.40±0.81^{a}
HCO_3^{-} (mmol/L)	19.97±0.09^{a}	20.27±0.07^{a}	19.90±0.15^{a}	20.03±0.09^{a}	19.87±0.18^{a}

Value is mean ± standard deviation at triplicate determination. (P<0.05). n=5

Table 3.4 Effects of Ethanolic leaf extract of *Combretum racemosum* on Serum Enzymatic Antioxidant Activities of lactating female Rats

Groups	Treatments	GPx (u/l)	SOD (u/l)	CAT (u/l)
1	Normal Rat Feed (Positive Control)	44.18±0.93^{b}	39.33±0.35^{b}	17.23±0.81^{b}
2	Lactating Rats (Negative Control)	38.29±0.83^{a}	29.23±1.23^{a}	14.30±0.48^{a}
3	Lactating rats+ 1000mg/kg of extract	41.16±1.07^{ab}	36.78±1.14^{b}	17.42±0.76^{b}
4	Lactating rat + 1500mg/kg of extract	41.96±0.70^{b}	38.47±0.50^{b}	17.26±0.43^{b}
5	Lactating rat + 2000mg/kg of extract	43.10±1.17^{b}	37.96±0.92^{b}	18.48±0.93^{b}

N=5 value is mean ± standard deviation. (P<0.05).

IV. Discussion

The body weight is the mass or quantity of heaviness of individual. Usually expressed by unit pound or kilogram. Table 3.1 above shows the average body of the experimental animals before and after the experiment as well as the percentage weight gain after the experimental period. This increase in weight is also envisaged owing to the fact that the animals were properly fed and other factors that necessitate growths were well taken care of. Group 2 (negative control) also showed a remarkable and significant percentage increase in weight. Group 3 - group 5 showed percentage increase in weight respectively. The decrease in percentage weight gain compared to the group 1 and group 2 could be as a result of combination of factors. One, the effect of the extract on the lactating animals. This factor can be validated as observed that the weight gain decrease down the group as the dose of the extract increases. The second factor could be traced to the stress associated with breastfeeding the young animals

Liver is an organ involved in many metabolic functions and is prone to xenobiotic induced injuries because of their central role in xenobiotic metabolism (Oyagbemin and Odetola, 2010)

The Liver contains a host of enzymes such as aspartate transaminase (AST), alanine transaminase (ALT) and alanine phosphatase (ALP). These enzymes are present in serum in very low concentration and have no known function in the serum other than provide insight about hepatic state and disorder (Chinyere *et al.,* 2015). The result obtained from this study (Table 3.2) suggested that there was significant increase ($p<0.05$) in the activities of ALP, AST and ALT when compered the normal control (Group 1) with negative control (Group 2). This significant increase in serum enzyme activities may however be an indication of cellular impairment that may lead to loss of functional integrity of the liver. Increase in hepatic enzyme activity observed maybe as a result of maternal autoimmune function recovery and immune clearance (Wong *et al*, 2022). But upon administration of the extract, the activities of the enzyme decreased significantly when compared the test groups (Group 3 – Group 5) with the negative control group (group 2). This is in agreement with the report of Okwuosa *et al,* (2020) that the leaf extract of *combretum racemosum* possess liver protective properties which justifies its use in folk medicine in South East Nigeria as a tonic. Total protein measures are rough measures of protein status but reflect major functional changes in liver integrity (Iweala and Osundiya, 2019). Total protein level from the present study was observed to decrease significantly in the group 2 (negative control) compared to group 1 (normal control). This decrease in total protein might be due to reduction in the functionality of the hepatocytes which in turn may have resulted in decrease in hepatic capacity to synthesize protein.

According to Iweala and Osundiya, (2019) decrease in protein is an indication of hepatocyte impairment possibly coursed by autoimmune diseases. Since total protein is synthesized by the liver cells, a decrease signifies an impaired hepatocellular function that could have consequences in overall physiological function of the animals. Shukla and Bhatia, (2010) also reported that decrease in total protein is often associated with increase in hepatic cell injury or inhibition of protein synthesis. Upon administration of the extract, the functional integrity of the hepatocytes was restored hence normal protein synthesis function was restored (Okwuosa *et al,* 2021). This was evident when compared the total protein of the test groups with the normal control group.

Albumin is the most abundant plasma protein synthesized in the liver, maintains water balance in the plasma and serum, transports and stores a wide variety of ligands (Akinlalu *et al*., 2016). Serum albumin has also been reported as a useful tool to assay the synthetic function of the liver (Abubakar *et al.,* 2019).

Result shows significant reduction in the albumin level of groups 2 compared to group 1. Significantly increased levels of albumin in groups treated with the extract compared to group two (negative control group) could further explain the hepatoprotective effect of the extract to the liver. This is also in agreement with the report of Okwuosa *et al,* (2020) that the leaf extract of *Combretum racemosum* possess liver protective properties.

Levels of bilirubin indicate the depth of jaundice induced by toxic substances (Mathew, 2021). Total bilirubin of the present study increased significantly ($p>0.05$) in group 2 compared to group 1. This significant increase in bilirubin level in group 2 could possibly be as a result of postpartum depression. (Yi-Liu *et al* 2024). But upon administration of the extract in the test rats, the level of bilirubin significantly decreased compared to the group two. Although the decrease was dose dependent on the test rats. This could be an indication that the extract may not induce jaundice. The kidneys perform the hemostasis function in the body. Urea and creatinine are indices of kidney function. Urea is the main end product of protein catabolism. The observed significant increase in urea and creatinine level in negative control group (group 2) when compared to the normal control group (group) could be associated to autoimmune and physiological changes associated with pregnancy and postpartum period. Urea level and creatinine level decreased significantly ($p<0.05$) in test rats against those of the negative control group though not in dose dependent ratio. This significant decrease may have been initiated by the test extract.

The retention of creatinine in the blood is evidence of kidney impairment. Creatinine level was significantly decreased in test rats when compared to those of the negative control group rats. Glutathione peroxidase (GPx), Superoxide dismutase (SOD) and Catalase (CAT) are antioxidant enzymes that synergistically neutralize plasma levels of O_2 and H_2O_2 in the body by suppressing oxygen radicals and mopping up organic peroxides formed as a result of exposure to oxidative stress (Ojo *et al.,* 2019). The results obtained from this study indicate that postpartum period as observed in group 2 led to significant decreased in GPx, CAT and SOD levels compared to group 1. This observation is line with the submission of karacor *et al* (2017) that pregnancy, labour and child birth are accompanied by excessive oxidative aggression. The excessive formation of free radicals (reactive oxygen species (ROS) and reactive nitrogen species (RNS), chloride reactive species (CRS) causes cellular damage); however, the administration ethanol leaf extract had a positive effect on these antioxidant enzymes as it restored their levels to normalcy. The perceived modulatory effects of *Combretum racemosum* leaf extract is indicative of their scavenging potentials. Data from this study agreed with the findings of Babatunde *et al.* (2024) who reported the scavenging activities of *Combretum racemosum*. Also, in a different but related work by Bhuija, (2020), he reported that methanolic leaf extract of *Combretum racemosum* showed high potentials of anti-oxidant activities.

V. Conclusion

Conclusively, this study has clearly demonstrated the protective tendency of *Combretum racemosum* ethanol leaf extract on hepatocellular, renocellular, and antioxidant potential on lactating rats.

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