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*Full Length Research Paper*

# Effect of *Theobroma cacao* on renal function of wistar albino rats induced with anaemia

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**The study investigated the effect of *Theobroma cacao* on renal function of phenylhydrazine induced anaemic albino rats. Forty albino rats were divided into 8 groups of five rats each namely control (group A), Phenyl hydrazine (group B) while groups C, D and E were given phenylhydrazine and administered with 100,200 and 500 mg/kg of *T. cacao* respectively while groups F, G and H were given 200,500 and 1000 mg/kg of *T cacao* only. The sodium, potassium, chloride, urea and creatinine were determined using Flame emission spectrophotometry, Mercuric nitrate, urease Berthelot and Jaffe's method respectively and subjected to statistical analysis using statistical package for social sciences (SPSS) version 18. There was significant difference (P<0.05) in sodium concentration (Mmol/l) of 130.60±2.74, 124.40±1.17, 130.00±1.40 and 131.73±1.26 in Control, Anaemia induced, Anaemia + *T. cacao* and *T. cacao* treated respectively while there was no significant difference (P>0.05) in Potassium (Mmol) concentrations of 4.35±0.96, 5.54±0.74, 6.14±0.30 and 5.38±0.53 in Control, Anaemia induced, Anaemia + *T. cacao and T. cacao* treated respectively. There was no significant difference in Chloride (Mmol/l) concentrations of 140.00±23.15, 137.60±14.84, 142.91±6.74 and 124.40±7.47 in control, anaemia induced, anaemia + *T. cacao* and *T. cacao* treated respectively. Urea concentrations (Mmol/l) of 2.60±0.81, 2.85±0.40, 2.58±0.29 and14.77±11.9547 in control, anaemia induced, anaemia + *T. cacao and T. cacao* treated did not show any significant difference as well as creatinine concentrations (umol/l) of 189.31±10.71, 155.18±10.25, 172.52±9.10 and 164.88±12.12 in control, anaemia induced, anaemia + *T. cacao* and *T. cacao* treated. The result of the study suggested that *T. cacao* extract caused no reversal in the renal dysfunction caused by phenylhrazine.**

**Key words:** Renal, *Theobroma cacao*, anaemia, phenylhydrazine.

## INTRODUCTION

The cocoa bean tree, *Theobroma cacao* Linnaeus belonging to family Sterculiaceae, originated from Latin America about 500 years ago, from where it was domesticated in other parts of the world. Harvested cocoa beans are usually fermented and dried prior to their being processed into finished products. Cocoa bean-beverages are processed products of the cocoa bean, sold under several brand names in Nigeria and worldwide. The nutraceutical values of raw cocoa bean products (RCBP) as well as the high acceptability of processed cocoa Bean-based beverages (PCB-BB), and their attractive flavor and appearance, designate the cocoa tree as a highly prized international cash crop. Chemical modifications of organic matters in cocoa bean occur through the processes of dextrinization, caramelization, pyrolysis, cyclization, oxidation and esterification reactions, which upon ingestion of the resultant organic derivatives may prompt tissue lesions in

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biologic systems. Claims for the medicinal capabilities of cocoa include, treatment of heart pain, shortness of breath, anaemia, burns, snakebite and wounds, angina, lowering of blood pressure, improving the efficiency of insulin action and anti-inflammatory properties amongst others. These medicinal properties have long been associated with the polyphenolic compounds which give flavor and color to chocolate.

Cocoa polyphenols (flavanols) have been reported to have a wide range of biological properties including modulating eicosanoid synthesis, increasing nitric oxide synthesis, lowering the rate of LDL-cholesterol oxidation, inhibiting platelets activation, stimulating the production of anti-inflammatory cytokines among others. By helping to protect tissues against stress, certain polyphenols work as preventive medicines for problems such as cardiovascular diseases, cancer, arthritis and autoimmune disorders (Xu and Zhao, 2004). They act as antioxidants due to their free radical scavenging properties, their ability to reduce the formation of free radicals and their ability to stabilize membrane by decreasing membrane fluidity. Among botanical medicines, cocoa, ginkgo, elderberry and green tea are examples of rich sources of antioxidant polyphenols. Some polyphenols (such as proanthocyanidins) exert beneficial cardiovascular effects through inhibition of platelet aggregation. Excess amounts of these polyphenols could theoretically extend blood clotting times. Examples of polyphenolic compounds present in cocoa are the flavan-3-ols or flavanols, which include the monomeric forms, (-) - epicatechin and (-) - catechin, and the oligomeric forms of the monomeric units, the procyanidins.

The liver and kidney are organs of homeostasis. The hepatic tissues play a central role in the biotransformation of xenobiotic and endogenous molecules prior to their elimination from the body. The biotransformation of xenobiotic in the hepatocytes may elicit the formation of noxious and highly reactive compounds or potentially toxic metabolites, which in the process of their metabolism predisposes the hepatocytes to injuries and dysfunction. The renal tissues are highly specialized in ensuring delicate balance in selective excretion or retention of body biomolecules, according to their physiologic renal threshold indices. The renal tissues are predisposed to chemical-induced injuries because of their action to concentrate tubular fluid by removal of $H_2O$, organic compounds and inorganic salts from the vascular system (Fapohunda and Afolayan, 2012). Liver (hepatic) function tests (LFT) and renal function test are diagnostic parameters for ascertaining organ integrity as well as functionality and level of recovery from pathologic injuries.

The prevalence and severity of anemia are related to the kidney disease stage (Abensur 2004; Kohagura et al., 2009) and the relative deficiency/reduction in erythropoietin (EPO) production is the main cause (Bastos et al., 2010; Canziani et al., 2006) because the kidneys produce this hormone that stimulates red blood cell production. In addition of EPO deficiency, other situations may contribute to the occurrence of anemia in chronic kidney disease (CKD), such as iron, folic acid and vitamin B12 deficiency; blood loss; hemolysis, hyperparathyroidism and inflammation, and these should be investigated before the introduction of EPO replacement therapy - the most common being iron deficiency (52.0%)(Abensur, 2004; Abensur et al., 2006).

This study aimed at determining the effect of bark extract of *Theobroma cacao* on renal function of albino rats induced with anaemia using sodium, potassium, chloride, urea and creatinine as indicators.

## MATERIALS AND METHODS

### Plant material

Mature bark of *T. cacao* was harvested from local farms in Njaba Local Government Area of Imo State, Nigeria and identified at the Department of Pharmacognosy of Madonna University.

### Animals

Forty Wistar albino rats were used for the biochemical studies. Wistar albino rats were purchased from the Animal House of the Faculty of Biological Sciences, University of Nigeria, Nsukka. The rats were fed rat pellets (Grand Cereals and Oil Mills Ltd, Jos, Nigeria) and water *ad libitum*.

### Preparation of plant material

The stem bark of *T. cacao* were removed from the cacao tree, and blanched immediately for 5 min at a temperature of 95°C, and air dried under a shed. The preparation of the stem bark of *T. cacao* was as described by Schinella et al. (2010). A known weight, 500 g of the stem bark was soaked in petroleum ether for three days for the purpose of defatting. The mixture was subsequently filtered using a muslin cloth. The residue was air-dried and extracted using a magnetic stirrer in hydroalcohol solvent of 70% ethanol for two hours. It was subsequently filtered using a muslin cloth and the solution was further filtered with Whatman no. 4 filter paper and the filtrate was concentrated to a semi-solid residue in a water bath at 60°C.

### Determination of stem bark content

The total stem bark content in *T. cacao* was determined using Folin-Ciocalteu's reagent as described by Velioglu et al. (1998) with slight modifications. The extract was prepared at a concentration of 1 mg/ml using ethanol. A measured volume, 100 µl, of the sample was mixed with 750 µl of Folin-Ciocalteu's Reagent (previously diluted 10-fold with distilled water) and allowed to stand for 5 min at a temperature of 25°C; $Na_2CO_3$ (0.57 M) solution (750 µl) was added to the mixture. After 90 min, the absorbance was measured using JENWAY 640 UV/VIS Spectrophotometer (Beckman/ Instruments Inc., Huston Texas) at 725 nm. Results were expressed as gallic acid equivalents (GAE) in milligram per 100 g dry weight of sample. The range of the calibration curve was from 0.01-0.1 mg/ml with R2 = 0.9588.

**Preparation of drug solutions**

Diphenylhydrazine used as the standard drug was weighed and dissolved in appropriate volume of distilled water. All solutions were kept in tightly closed sterile bottles and were made use of on the same day. Leftovers were discarded.

**Induction of anaemia**

Anaemia was induced by intraperitoneal injection of diphenylhydazine (60 mg/kg body weight), dissolved in physiological saline, for 2 consecutive days in accordance with the methods of Rona et al. (1959) and Seth et al.(1998). Anaemia was allowed to establish in 24 h after the second induction. Packed cell volume<35% was considered as an index for anaemia.

**Experimental design**

Forty Wistar rats housed together and fed normal rat feed and water *ad libitum* under hygienic condition for a period of 40 days were randomly divided into 8 groups of 4 rats. The Group 1 was given rat diets and water *ad libitum* to serve as control while Group 2 was given di phenyl hydrazine (PHZ) to induce anaemia. Group 3 was given PHZ to induced anaemia and administered with 100 mg/kg b.w of TBC, Group 4 was given PHZ to induced anaemia and administered with 200 mg/kg b.w of TBC while Group 5 was given PHZ to induced anaemia and administered with 500 mg/kg b.w of TBC. Group 6 albino rats were treated with 200 mg/kg b.w of TBC, Group 7 albino rats were treated with 500 mg/kg b.w of TBC while Group 8 albino rats were treated with 1000 mg/kg b.w of TBC respectively for 14 days. The route of administration of the bark extract of cacao was by oral intubation. At the end of treatment, the animals were then sacrificed. Blood were collected into sample bottles from the heart. The animals were sacrificed by medial decapitation along the stomach and blood was collected from the heart, transferred to plain test tubes, allowed to clot and subsequently centrifuged to obtain the serum component which was used for further biochemical analysis.

**Biochemical assay**

The sodium and potassium estimation was done using flame photometric method as described by Baker et al. (1998) using Gallenkamp flame photometer. Using compressed air, diluted (1 in 10) serum was sprayed as a fine mist of droplets (Nebulised) into a non luminous gas flame which becomes coloured by the characteristic mission of the sodium or potassium metallic ions in the sample. Light of a wavelength corresponding to the metal being measured was selected by a light filter or prism system and allowed to fall on a photosensitive detector system. The amount of light emitted depends on the concentration of metallic ions present. Accuracy was controlled by analyzing a sodium/potassium standard solution (140/3.0 mmol/l) respectively after every two analysis to correct for instrument drift while a Randox normal quality control serum was assayed to determine the precision.

Urea estimation was done by Urease - Berthelot colorimetric method. Ten microlitre of sample, standard, control and distilled water was pipette into test tube labeled sample, standard control and blank respectively. Hundred microlitre of urea reagent 1 was added to all the tubes and incubated at 37°C for 10 min. 250 µl of urea solutions 2 and 3 was added to all the tubes, mixed and incubated at 37°C for 15 min. The absorbance of the sample, control and standard were read at 546 nm against the content of the blank tube. The activity of sample was calculated using the absorbance of sample against absorbance of standard multiplied by concentration of standard (Weatherburn, 1967).

Creatinine estimation was done by Jaffe's colorimetric method. Five hundred millilitre of sample, standard, control and distilled water was pipette into test tube labeled sample, standard control and blank respectively containing five hundred millilitre of trichloroacetic acid (TCA).The contents were mixed and spun at 2500 rpm for 10 min. 1000 ml of supernatant from each tube was added into respectively labeled test tube containing 1000 ml of reagent mixture of Picric acid and sodium hydroxide (500 ml each). The contents were mixed and stand at 25°C for 20 min. The absorbance of the sample, control and standard were read at 546 nm against the content of the blank tube. The concentration of sample was calculated using the absorbance of sample against absorbance of standard multiplied by concentration of standard (Henry, 1974).

**Statistical analysis**

The biochemical data were subjected to some statistical analysis as the Mean (X), standard error of mean (SEM) and ANOVA using Statistical Package for Social Sciences (SPSS) version 18.

## RESULTS

The sodium concentration (Mmol/l) was 136.00±2.74, 124.40±1.17, 130.50±2.06, 128.50±1.26, 131.33±4.67, 132.20±2.20,130.20±2.03 and 132.80±2.58 in control, anaemia, anaemia+100 mg *T. cacao,* anaemia+200 mg *T. cacao,* anaemia+500 mg *T. cacao,* 200 mg *T. cacao,* 500 mg *T. cacao* and 1000 mg *T. cacao* respectively. The potassium concentration (Mmol/l) was 4.35±0.96, 5.53±0.74, 6.45±0.79, 5.90±0.35, 6.05±0.11, 6.88±0.82, 4.59±0.50 and 4.68±1.08 in control, anaemia, anaemia+100 mg *T. cacao,* anaemia+200 mg *T. cacao*, anaemia+500 mg *T. cacao*, 200 mg *T. cacao*, 500 mg *T. cacao* and 1000 mg *T. cacao* respectively. The chloride concentration (Mmol/l) was 140.00±23.15, 137.60±14.84, 157.00±8.54, 141.00±14.27,126.67±3.53, 135.20±19.12, 125.60±8.16 and 112.40±9.39 in control, anaemia, anaemia+100 mg *T. cacao,* anaemia+200 mg *T. cacao,* anaemia + 500 mg *T. cacao*, 200 mg *T. cacao*, 500 mg *T. cacao* and 1000 mg *T. cacao* respectively. The urea concentration (Mmol/l) was 2.60±0.81, 2.85±0.40, 2.23±0.40, 2.96±0.72, 2.55±0.10, 3.10±0.95, 2.18+0.33 and 39.02±35.75 in control, anaemia, anaemia+100 mg *T. cacao*, Anaemia+200 mg *T. cacao,* Anaemia+500 mg *T. cacao,* 200 mg *T. cacao*, 500 mg *T. cacao* and 1000 mg *T. cacao* respectively. The creatinine concentration (Umol/l) was 189.31±10.71, 155.18±10.25, 162.46±9.30, 188.06±21.79, 165.20±11.80, 177.81±13.00, 122.13±8.11 and 194.70±24.24 in control, anaemia, anaemia+100 mg *T. cacao,* anaemia+200 mg *T. cacao,* Anaemia+500 mg *T. cacao,* 200 mg *T. cacao,* 500 mg *T. cacao* and 1000 mg *T. cacao* respectively as shown in Table 1.

Table 2 shows the comparison between the mean values of serum electrolyte for sodium, potassium, chloride, urea, and creatinine in the serum of Wistar albino rats administered with graded doses of bark

**Table 1.** Effect of different doses of *T.cacao* on renal function of anaemia induced albino rats.

| Group | sodium (Mmol/l) | Potassium (Mmol/l) | Chloride (Mmol/l) | Urea (Mmol/l) | Creatinine (umol/l) |
|---|---|---|---|---|---|
| Control | 136.00±2.74 | 4.35±0.96 | 140.00±23.15 | 2.60±0.81 | 189.31±10.71 |
| Anaemia | 124.40±1.17 | 5.53±0.74 | 137.60±14.84 | 2.85±0.40 | 155.18±10.25 |
| Anaemia+100 mg *T. cacao* | 130.50±2.06 | 6.45±0.79 | 157.00±8.54 | 2.23±0.40 | 162.46±9.30 |
| Anaema+200 mg *T. cacao* | 128.50±1.26 | 5.90±0.35 | 141.00±14.27 | 2.96±0.72 | 188.06±21.79 |
| Anaemia+500 mg *T. cacao* | 131.33±4.67 | 6.05±0.11 | 126.67±3.53 | 2.55±0.10 | 165.20±11.80 |
| 200 mg *T. cacao* | 130.20±2.03 | 6.88±0.82 | 135.20±19.12 | 3.10±0.95 | 177.81±13.00 |
| 500 mg *T. cacao* | 132.20±2.20 | 4.59±0.50 | 125.60±8.16 | 2.18±0.33 | 122.13±8.11 |
| 1000 mg *T. cacao* | 132.80±2.58 | 4.68±1.08 | 112.40±9.39 | 39.02±35.75 | 194.70±24.24 |
| F | 2.239 | 1.451 | 0.842 | 0.855 | 2.58 |
| P | 0.062 | 0.227 | 0.563 | 0.553 | 0.032 |
| **Post hoc** | | | | | |
| Control vs. anaemia | 0.164 | 0.998 | 1.000 | 1.000 | 0.544 |
| Control vs. anaemia + 100 mg *T. cacao* | 0.881 | 0.849 | 1.000 | 1.000 | 0.755 |
| Control vs. anaemia + 200 mg *T. cacao* | 0.493 | 0.894 | 1.000 | 1.000 | 1.000 |
| Control vs. anaemia + 500 mg *T. cacao* | 0.998 | 0.796 | 1.000 | 1.000 | 0.905 |
| Control vs. 200 mg *T. cacao* | 0.845 | 0.697 | 1.000 | 1.000 | 1.000 |
| Control vs. 500 mg *T. cacao* | 0.994 | 1.000 | 1.000 | 1.000 | 0.037 |
| Control vs. 1000 mg *T. cacao* | 1.000 | 1.000 | 0.986 | 0.993 | 1.000 |
| Anaemia vs. control | 0.164 | .998 | 1.000 | 1.000 | 0.544 |
| Anaemia vs. anaemia + 100 mg *T. cacao* | 0.447 | 1.000 | 0.991 | 0.994 | 1.000 |
| Anaemia vs. anaemia + 200 mg *T. cacao* | 0.501 | 1.000 | 1.000 | 1.000 | 0.945 |
| Anaemia vs. anaemia + 500 mg *T. cacao* | 0.897 | 1.000 | 1.000 | 1.000 | 1.000 |
| Anaemia vs. 200 mg *T. cacao* | 0.465 | 0.987 | 1.000 | 1.000 | 0.964 |
| Anaemia vs. 500 mg *T. cacao* | 0.240 | 0.996 | 1.000 | 0.976 | 0.426 |
| Anaemia vs. 1000 mg *T. cacao* | 0.297 | 1.000 | .944 | 0.994 | 0.916 |
| Anaemia + 100 mg T. cacao vs. control | 0.881 | 0.849 | 1.000 | 1.000 | 0.755 |
| Anaemia + 100 mg *T. cacao* vs. anaemia | 0.447 | 1.000 | 0.991 | 0.994 | 1.000 |
| Anaemia + 100 mg *T. cacao* vs. anaemia + 200 mg *T. cacao* | 1.000 | 1.000 | 0.997 | 0.999 | 0.989 |
| Anaemia + 100 mg *T.cacao* vs. anaemia + 500 mg *T. cacao* | 1.000 | 1.000 | 0.271 | 0.999 | 1.000 |
| Anaemia + 100 mg *T. cacao* vs. 200mg *T.cacao* | 1.000 | 1.000 | 0.995 | 0.999 | 0.999 |
| Anaemia + 100 mg *T. cacao* vs. 500mg *T.cacao* | 1.000 | 0.714 | 0.381 | 1.000 | 0.199 |
| Anaemia + 100 mg *T. cacao* vs. 1000mg *T.cacao* | 1.000 | 0.970 | 0.142 | 0.992 | 0.975 |
| Anaemia + 200 mg *T. cacao* vs. control | 0.493 | 0.894 | 1.000 | 1.000 | 1.000 |

**Table 1.** Contd.

| | | | | | |
|---|---|---|---|---|---|
| Anaemia + 200 mg *T. cacao* vs. anaemia | 0.501 | 1.000 | 1.000 | 1.000 | 0.945 |
| Anaemia + 200mg *T. cacao* vs. anaemia + 100 mg *T. cacao* | 1.000 | 1.000 | 0.997 | 0.999 | 0.989 |
| Anaemia + 200mg *T.cacao* vs. anaemia + 500 mg *T. cacao* | 1.000 | 1.000 | 0.994 | 1.000 | 0.998 |
| Anaemia + 200 mg *T. cacao* vs. 200 mg *T.cacao* | 1.000 | 0.991 | 1.000 | 1.000 | 1.000 |
| Anaemia + 200 mg *T. cacao* vs. 500 mg *T.cacao* | 0.934 | 0.634 | 0.998 | 0.995 | 0.387 |
| Anaemia + 200 mg *T. cacao* vs. 1000 mg *T.cacao* | 0.919 | 0.992 | 0.852 | 0.994 | 1.000 |
| Anaemia + 500 mg *T. cacao* vs. control | 0.998 | 0.796 | 1.000 | 1.000 | 0.905 |
| Anaemia + 500 mg *T. cacao* vs. anaemia | 0.897 | 1.000 | 1.000 | 1.000 | 1.000 |
| Anaemia + 500mg *T. cacao* vs. anaemia + 100 mg *T.cacao* | 1.000 | 1.000 | 0.271 | 0.999 | 1.000 |
| Anaemia + 500 mg *T.cacao* vs. anaemia + 200 mg *T.cacao* | 1.000 | 1.000 | 0.994 | 1.000 | 0.998 |
| Anaemia + 500 mg *T.cacao* vs. 200 mg *T.cacao* | 1.000 | 0.994 | 1.000 | 1.000 | 1.000 |
| Anaemia + 500 mg *T.cacao* vs. 500 mg *T.cacao* | 1.000 | 0.373 | 1.000 | 0.992 | 0.336 |
| Anaemia + 500 mg *T.cacao* vs. 1000 mg *T.cacao* | 1.000 | 0.965 | 0.936 | 0.993 | 0.992 |
| 200 mg *T. cacao* vs. control | 0.845 | 0.697 | 1.000 | 1.000 | 1.000 |
| 200 mg *T. cacao* vs. anaemia | 0.465 | 0.987 | 1.000 | 1.000 | 0.964 |
| 200 mg *T. cacao* vs. anaemia + 100mg *T.cacao* | 1.000 | 1.000 | 0.995 | 0.999 | 0.999 |
| 200 mg *T.cacao* vs. anaemia + 200mg *T.cacao* | 1.000 | 0.991 | 1.000 | 1.000 | 1.000 |
| 200 mg *T.cacao* vs. anaemia + 500mg *T.cacao* | 1.000 | 0.994 | 1.000 | 1.000 | 1.000 |
| 200 mg *T.cacao* vs. 500mg *T.cacao* | 1.000 | 0.510 | 1.000 | 0.999 | 0.129 |
| 200 mg *T.cacao* vs. 1000mg *T.cacao* | 1.000 | 0.887 | 0.994 | 0.994 | 1.000 |
| 500 mg *T.cacao* vs. control | 0.994 | 1.000 | 1.000 | 1.000 | .037 |
| 500 mg *T.cacao* vs. anaemia | 0.240 | 0.996 | 1.000 | 0.976 | 0.426 |
| 500 mg *T.cacao* vs. anaemia + 100mg *T.cacao* | 1.000 | 0.714 | 0.381 | 1.000 | 0.199 |
| 500 mg *T.cacao* vs. anaemia + 200mg *T.cacao* | 0.934 | 0.634 | 0.998 | 0.995 | 0.387 |
| 500 mg *T.cacao* vs. anaemia + 500mg *T.cacao* | 1.000 | 0.373 | 1.000 | 0.992 | 0.336 |
| 500 mg *T.cacao* vs. 200mg *T.cacao* | 1.000 | 0.510 | 1.000 | 0.999 | 0.129 |
| 500 mg *T.cacao* vs. 1000mg *T.cacao* | 1.000 | 1.000 | 0.997 | 0.992 | 0.354 |
| 1000 mg *T.cacao* vs. control | 1.000 | 1.000 | 0.986 | 0.993 | 1.000 |
| 1000 mg *T.cacao* vs. anaemia | 0.297 | 1.000 | 0.944 | 0.994 | 0.916 |
| 1000 mg *T.cacao* vs. anaemia + 100 mg *T.cacao* | 1.000 | 0.970 | 0.142 | 0.992 | 0.975 |
| 1000 mg *T.cacao* vs. anaemia + 200 mg *T.cacao* | 0.919 | 0.992 | 0.852 | 0.994 | 1.000 |
| 1000 mg *T.cacao* vs. anaemia + 500 mg *T.cacao* | 1.000 | 0.965 | 0.936 | 0.993 | 0.992 |
| 1000 mg *T.cacao* vs. 200 mg *T.cacao* | 1.000 | 0.887 | 0.994 | 0.994 | 1.000 |
| 1000 mg *T.cacao* vs. 500 mg *T.cacao* | 1.000 | 1.000 | 0.997 | 0.992 | 0.354 |

**Table 2.** Effect of *T. cacao* on renal function of anaemia induced albino rats.

| Group | Sodium (Mmol/l) | Potassium (Mmol/l) | Chloride (Mmol/l) | Urea (Mmol/l) | Creatinine (umol/l) |
|---|---|---|---|---|---|
| Control | 130.60±2.74 | 4.35±0.96 | 140.00±23.15 | 2.60±0.81 | 189.31±10.71 |
| Anaemia induced | 124.40±1.17 | 5.54±0.74 | 137.60±14.84 | 2.85±0.40 | 155.18±10.25 |
| Anaemia + *T. cacao* | 130.00±1.40 | 6.14±0.30 | 142.91±6.74 | 2.58±0.29 | 172.52±9.10 |
| *T. cacao* | 131.73±1.26 | 5.38±0.53 | 124.40±7.47 | 14.77±11.95 | 164.88±12.12 |
| F | 5.17 | 1.140 | 0.920 | 0.430 | 0.710 |
| P | 0.010 | 0.340 | 0.440 | 0.730 | 0.550 |
| **Post Hoc** | | | | | |
| Control vs. anaemia induced | 0.070 | 0.885 | 1.000 | 1.000 | 0.242 |
| Control vs. anaemia + *T. cacao* | 0.405 | 0.497 | 1.000 | 1.000 | 0.785 |
| Control vs. *T. cacao* | 0.667 | 0.897 | 0.977 | 0.882 | 0.591 |
| Anaemia induced vs. Control | 0.070 | 0.885 | 1.000 | 1.000 | 0.242 |
| Anaemia induced vs. anaemia + *T. cacao* | 0.048 | 0.959 | 0.999 | 0.992 | 0.746 |
| Anaemia induced vs. *T. cacao* | 0.005 | 1.000 | 0.950 | 0.890 | 0.988 |
| Anaemia + *T. cacao* vs. Control | 0.405 | 0 | 1.000 | 1.000 | 0.785 |
| Anaemia + *T. cacao* vs. anaemia induced | 0.048 | 0.959 | 0.999 | 0.992 | 0.746 |
| Anaemia + *T. cacao* vs. *T. cacao* | 0.923 | 0.762 | 0.367 | 0.880 | 0.996 |
| *T. cacao* vs. Control | 0.667 | 0.897 | 0.977 | 0.882 | 0.591 |
| *T. cacao* vs. Anaemia induced | 0.005 | 1.000 | 0.950 | 0.890 | 0.988 |
| *T. cacao* vs. anaemia + *T. cacao* | 0.923 | .762 | 0.367 | 0.880 | 0.996 |

extract of *T. cacao* after induction of anaemia. There was significant difference ($p<0.05$) in sodium of the serum of untreated group when compared with the *T. cacao* group. All the groups treated with the extract doses showed significant increase ($p>0.05$) when compared with the untreated group apart from urea. However, no significant change ($p >0.05$) was observed in the serum potassium, chloride, creatinine and Urea in the test when compared with the control.

## DISCUSSION

The result of this study showed that sodium, chloride and creatinine concentrations decrease with increase in potassium and urea concentration in anaemic induced albino rats compared with their respective controls. This is suggestive that albino rats induced with anaemic had changes in renal parameters. This is similar to study by Carl et al. (2015). Potassium homeostasis is maintained predominantly through regulation of renal excretion. Cocoa increased the Na+-k+ ATPase as it increased the performance of this membrane bound enzymes due to alteration of electrolyte. Kang et al. (2006) under normal physiologic conditions, urea is the primary vehicle for the excretion of metabolic nitrogen, whose sources are, for the most part, traceable to dietary constituents and body protein turnover. Urea is a low threshold substance, which is why it is rapidly cleared from vascular system by the renal system. Therefore, raised level of urea nitrogen concentration in blood is diagnostic of renal dysfunction.

Also the result of this study showed a dose dependent increase in sodium, potassium, chloride with exception at 500 mg/kg and creatinine concentrations with dose dependent decrease in urea concentration with exception at 200 mg/kg in albino rats induced with anaemia treated with different doses of *T. cacao* compared with the anaemic induced albino rats. Furthermore, Kang et al. (2006) had earlier noted significant elevation of serum urea concentration against marginal alterations of serum creatinine concentration in streptozotocin-induced diabetic rats that exhibited renal dysfunction, which conformed to the present findings. Creatinine is sourced from the muscle protein turnover, and urinary creatinine concentration is proportionate to muscle mass and remains relatively constant. However, increase in serum creatinine concentration can result from increased ingestion of cooked meat than *T. cacao* but there was significant difference ($p<0.05$) in creatinine treated with 500mg T. Cacao group when compared with the control group. All the groups treated with the extract doses (200, 500 and 1000 mg) showed significant increase ($p<0.05$) when compared with the untreated group (Table 1).

The result of the study further showed administration of *T. cacao* affected sodium concentrations while there were no significant effects on chloride, creatinine, potassium and urea concentrations in anaemic induced albino rats. This is contrasting with study by Kosoko et al. (2017) who

reported that high flavonoids of *T. cacao* inhibit general toxicity, renal and splenic damage caused by Doxorubicin. This is suggestive that *T. cacao* administration did not reverse the renal damage caused by Phenylhydrazine.

## Conclusion

The result has shown that there was an increase in sodium concentration in *T. cacao* treated rats compared to the anaemic induced rats but overall *T. cacao* administration did not reverse the renal damage caused by phenylhydrazine.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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