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African Journal of Cellular Pathology

Full Length Research Paper

Some plasma biochemical changes in layers experimentally infection with *Pasteurella multocida*

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The present study was carried out to investigate some biochemical alterations in layers experimentally infected with *Pasteurella multocida*. A total of 20 eighteen-week old ISA Brown layers were used in the experiment. The birds were randomly assigned to two groups (infected and control) of 10 layers each. To establish infection, each bird in the infected group was challenged by intra nasal (0.1 ml) and intramuscular (0.4 ml) administration of *P. multocida* inoculum containing 4.5 × 10^8 CFU/ml. Meanwhile, birds in the control group were given clean drinking water and fed standard commercial layers mash *ad libidum.* All the experimental birds were monitored closely for clinical signs of fowl cholera. Blood samples were collected from both groups at day zero (Day 0), 2, 4, 7, 14, 21, 28, 35, 42, post-infection (pi) and used to assay some biochemical parameters. By day 5 post-inoculation (pi), all birds in the infected group manifested clinical signs typical of fowl cholera; weakness, ruffled feathers, sneezing, greenish-yellowish diarrhoea, decrease in feed and water consumption, weight loss, drop in egg production and mortality rate of (20%). However, there were significant increase in the plasma activities of aspartate amino transferase, alanine aminotransferase, alkaline phosphatase, and level of uric acid and significant hypoproteinaemia. The experimental *P. multocida* infection initiated hepatic, intestinal and renal dysfunctions.

Key words: *Pasteurella multocida*, cholera, ISA brown layers.

INTRODUCTION

Fowl cholera (avian pasteurellosis) is a contagious and economically important disease of poultry caused by a Gram negative, non-motile fermentative organism, *Pasteurella multocida* (Christensen and Bisgaard, 2000). Beside chickens, turkeys, ducks and geese, all other types of birds are also susceptible to the disease (Glisson et al., 2003). The disease can affect birds of any age, but it rarely occurs in commercial poultry of less than 8 weeks of age (Rimler and Rhoades, 1994). It causes devastating economic losses to the poultry industry

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through weight loss condemnations of carcasses and death world-wide (Aye et al., 2001). It has been reported to cause 1.8-21% mortality and a decline in egg production by 15-20% and resulted in shorter egg laying period leading to the infected flocks being culled at an earlier stage (Compi et al., 1990; Kempentanov et al., 2000). The disease remains a significant obstacle due to losses to commercial poultry production in most part of tropical Asia and Africa. It usually occurs as a fulminating disease with massive bacteraemia, high morbidity and mortality (OIE, 2008). Fowl cholera is one of the diseases known to affect and produce some pathological changes like oophoritis, regression of the ovary, serofibrinous pericarditis and catarrhal tracheitis with excessive mucus (Rosales, 2013).

In Nigeria, intensive large and medium scale poultry production has grown tremendously over the past two decades giving rise to numerous challenges (Hassan et al., 2006). However, family poultry production continues to make a significant contribution to the poverty alleviation and house hold food security in many developing countries (Alders and Pym, 2009; Gueye, 2012). The main challenge in raising chickens is the large economic losses due to various diseases prominent among which is fowl cholera (Davis-West, 1972).

Three clinical forms of the disease in poultry have been identified; namely, per- acute, acute and chronic forms. The per-acute form is associated with the most virulent and highly infectious organism; birds in good conditions are suddenly found dead with no premonitory signs. In the acute form, chickens will show anorexia, mucus discharge from the beak, high fever, loss of weight, drop in egg production, cyanosis of wattles and comb, green foetid diarrhoea (Rosales, 2013).The chronic form of the disease is associated with conjunctivitis, swollen wattles, tracheitis, lameness, and dyspnoea, swelling of joints and tendon sheaths of legs and wings, torticollis (Gustafson et al., 1998).

Gross lesions in chickens include petechial and ecchymotic haemorrhages on coronary fats of the heart, proventriculus, gizzard, peritoneum, intestines, and abdominal fats occur. The liver is frequently enlarged, congested streaked with multiple pinhead greyish necrotic foci and there is splenomegaly and congestion of ovarian follicles (Abdu, 2014). The mode of transmission of the disease can be by mechanical means through vectors, aerosol and ingestion of contaminated feed and water. Most farm animals may be carriers of *P. multocida* (Blackall, 2003).

MATERIALS AND METHODS

Study Area

This study was carried out in the Department of Veterinary Pathology, Faculty of Veterinary Medicine, Ahmadu Bello University Samaru, Zaria, which is located within the Northern Guinea Savannah Zone of Nigeria, between latitude 7° and 11° North and longitude 7° and 44°E; the average rainfall of this zone ranges from 1,000 to 1,250 mm and the average temperature ranges from 17 to 33° C (Sa'idu et al., 1994).

Experimental Birds and Housing

A total of 20 eighteen-week-old ISA Brown layers, immunized against all vaccinable endemic diseases other than fowl cholera, were acquired from a reputable farm that brooded poultry for research purposes at Kujama, Kaduna State. On arrival at the Poultry Research Unit of the Department of Pathology in the Faculty of Veterinary medicine, they were housed and managed intensively in pens that were thoroughly washed and sprayed with disinfectant. The birds were kept for 7 weeks to acclimatize to the new environment and handling conditions.

Source of bacterial organism

The challenge bacterium, *P. multocida* serotype A: 1, used in this study was provided by the Department of Bacteriology, National Veterinary Research Institute, Vom, Plateau State, Nigeria.

Sub-culture of organism

The bacterium on the slant was sub-cultured on blood agar and incubated at 37°C for 24 h. The resulting colonies that have similar characteristics with *P. multocida* sero-type A:1 were then subjected to biochemical tests (indole and sugar tests) according to standard procedures described by Glisson et al. (2008). Bacterial inoculum was prepared using McFarland Standards, which were turbidity standards used to approximate the concentration of bacteria in a liquid suspension as reviewed by Acharya (2016). In the laboratory McFarland turbidity Standards were prepared by mixing a 1% solution of anhydrous Barium chloride plus 1% solution of sulphuric acid to obtain a barium precipitate after which the volumes of the two reagents were adjusted to prepare standards of different turbidities that represent different concentrations of bacterium. The turbidity of a suspension of bacteria was visually compared using the standards.

Pre-inoculation bacteriological monitoring of experimental birds

Prior to commencement of the experiment, nasal swabs were collected from all the experimental birds and were used to inoculate an already prepared blood agar and MacConkey agar after which they were incubated at 37°C for 24 h. The growth (colonies) on the blood agar plates were then subjected to biochemical tests (indole and sugar tests) according to standard laboratory procedures described by Glisson et al. (2008).

Inoculation of birds with *P. multocida*

After reaching peak of egg production (80%) at 26 weeks old, the birds were assigned at a random into two groups (infected and control) of 10 layers each. several colonies from serotype A:1 were scooped from the blood agar and put into a single test-tube, containing 20 ml of 0.5% normal saline and mixed till when the turbidity was equivalent to 9×10^8 CFU/ML which is standard 3 from McFarland standards. On the day of infection (Day 0) each of the birds in the infected group was challenged with dose of 0.5 ml of the inoculum containing 4.5×10^8 CFU/ML of *P. multocida.* One

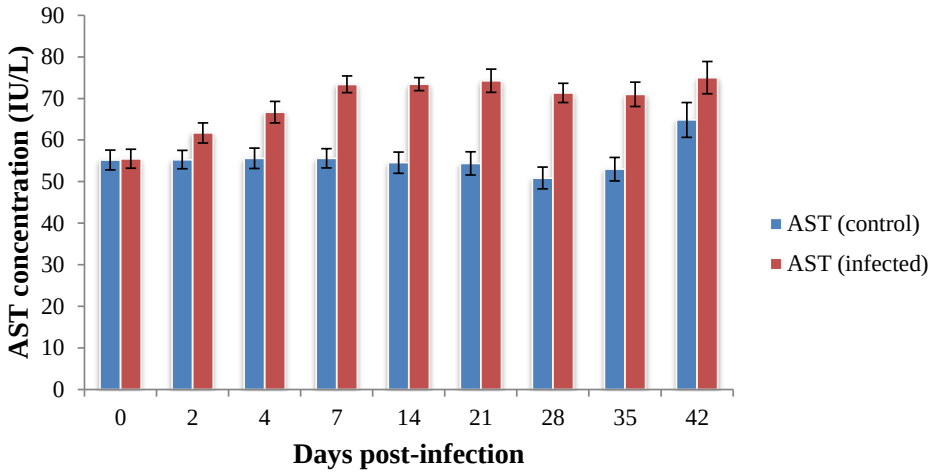

Figure 1. Mean (± SEM) aspartate aminotransferase (AST) concentrations of *P. multocida* experimentally-infected and control groups of layers.

tenth (0.1 ml) and 0.4 ml of the solution were administered intra-nasally (Arsov, 1965) and intra-muscularly (Amany and Abd-Alla, 1997) respectively to each bird using insulin syringe.

Determination of plasma biochemical parameters

Blood samples of 2.5 ml was collected from each of the birds in the infected and control groups via the brachial vein, using 5-ml syringe and 23 G needle, on day 0 2, 4, 7, 14, 21, 28, 35 and 42 pi. The blood was dispensed into EDTA sample bottles which were used for plasma biochemical analyses of the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), levels of total proteins and uric acid at Chemical Pathology Laboratory, Ahmadu Bello University Teaching Hospital, Shika, Zaria.

Statistical analysis

All the data obtained were subjected to statistical analysis including the calculation of the means and standard error of the means. Data between groups were compared with Student t-test and values of $P < 0.05$ were considered significant using Graph Pad Prism Version 5.00 for Windows, Graph Pad Software, San Diego California USA.

RESULTS

Results of the bacteriological monitoring pre-infection showed that all the experimental birds in both groups tested negative to fowl cholera disease before the commencement of the experiment.

Clinical manifestation of fowl cholera disease in the infected layers

Following infection with *P. multocida* serotype A:1, birds in the infected group appeared clinically normal until day 5 pi when the birds started to show the typical clinical signs of fowl cholera, which included drop in egg production, reduced feed and water consumption, weakness, ruffled feathers, watery greenish-yellowish faeces and weakness, loss of weight and laboured breathing, and later followed included paleness and cyanosis of wattles and combs. During the experiment 20% mortality rate was recorded in the infected group on days 14 and 28 pi respectively, while two other birds that showed clinical signs of fowl cholera were selected and necropsied aseptically. Throughout the experiment period all the birds in the control group appeared apparently healthy.

Recovery of bacterial organism from infected birds

At necropsy, swabs were aseptically taken from the blood in the hearts, lungs and spleen of the birds in the infected group for the isolation of *P. multocida* starting from day 7 and throughout the period of experiment. Biochemical test carried in the laboratory revealed nitrate was reduced; indole and hydrogen sulphide were produced, while methyl red and Voges–Proskauer tests were negative.

Effect of *P. multocida* Infection on some biochemical parameters in layers

Mean plasma aspartate aminotransferase activity

The mean plasma aspartate amino transferase (AST) activity in the infected and control groups are as shown in Figure 1. Mean plasma AST activity in the infected group increased significantly ($P < 0.05$) from day 2 post-

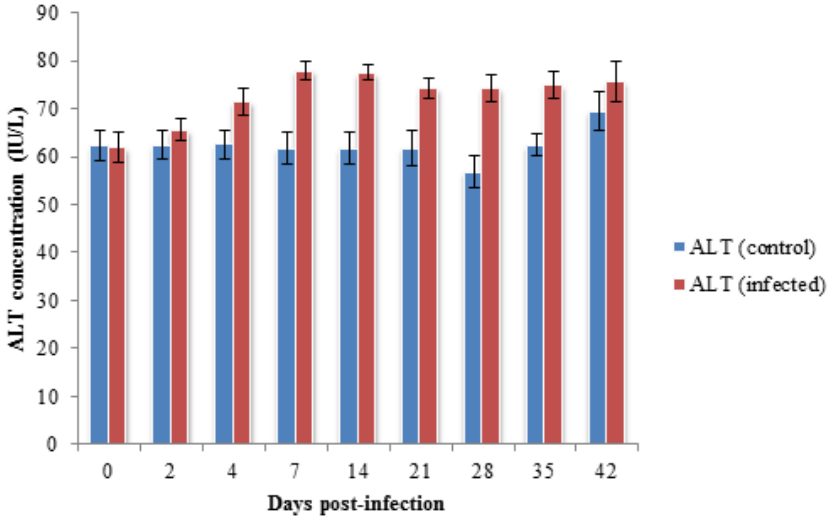

Figure 2. Mean (± SEM) plasma alanine aminotransferase (ALT) concentrations of *P. multocida* infected and control groups in layers.

infection (pi) to reach a peak level (75.00 ± 3.89 IU/L) on day 42 pi when compared with that in the control group (65.83 ± 4.21 IU/L).

Mean plasma alanine aminotransferase activity.

The mean plasma alanine aminotransferase activity (ALT) in the infected and control groups are as shown in Figure 2. Progressive increase ($P < 0.05$) in mean ALT activity was observed in the infected group and this was significantly ($P < 0.05$) higher than that recorded in the control group beginning from day 4 with peak level (78.00 ± 1.89 IU/L) attained on day 7 pi. Thereafter, the mean ALT activity of the infected group remained relatively unchanged until termination of the experiment (day 42 pi).

Mean plasma alkaline phosphatase activity

The mean plasma alkaline phosphatase activity (ALP) in the infected and control groups are shown in Figure 3. Mean plasma alkaline phosphatase activity showed no change in both the infected and control groups initially before it increased significantly to a highest level (4.293 ± 9.53 IU/L) on day 4 pi in the infected group of birds. On day 7 pi the level was still high in the infected before decreasing gradually from day 14 to 42 pi when no significant difference were observed between the two groups of birds.

Mean plasma urea concentration

The mean plasma urea concentration in the infected and control groups are shown in Figure 4. The infection caused significant increase ($P < 0.05$) in mean plasma urea concentration in the infected group on day 7 pi with highest level (7.17 ± 0.55 mg/dl) reached on day 14 pi. It then decreased progressively until termination of the experiment on day 42 pi.

Plasma total protein concentration

The mean plasma total protein concentration in the infected and control groups are shown in Figure 5. Mean plasma total protein concentration significantly increase ($P < 0.05$) in the infected from day 7 pi to reach a highest level (8.70 ± 0.12 mg/dl) on day 14 pi which later dropped to a lowest level (5.50 ± 0.21 mg/dl) on day 35 pi until termination of the research on day 42 pi.

DISCUSSION

The clinical signs that manifested in the *P. multocida*-infected layers during the experiment included anorexia, weakness, loss of weight, greenish-yellowish diarrhoea, drop in egg production, pale wattles and combs which are in agreement with those previously reported by Christensen (2013). The observed significant ($P < 0.05$) increase in plasma activities of ALT, AST and ALP in the *P. multocida*-infected layers suggests hepatic, renal, and gastrointestinal lesions that had occurred consequent to possible effect of endotoxins that might have been released by the infecting organism or due to cytopathiec effect of the organism which are in line with the findings by Bokori and Karasi (1969). The significant ($P < 0.05$) increase in the mean plasma urea concentration noticed

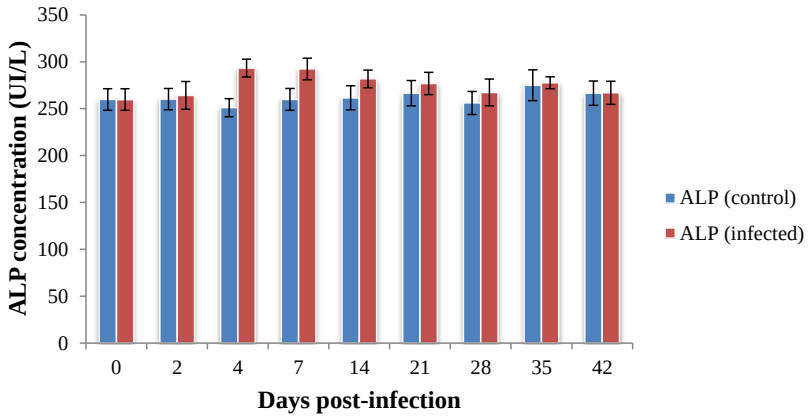

Figure 3. Mean (± SEM) Alkaline phosphatise (ALP) concentrations of *P. multocida* infected and control groups in layers

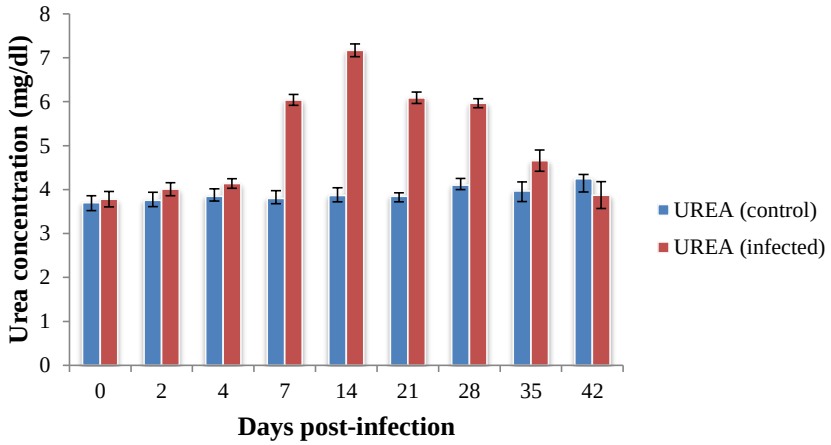

Figure 4. Mean (± SEM) plasma urea concentrations of *P. multocida* experimentally infected and control groups in layers

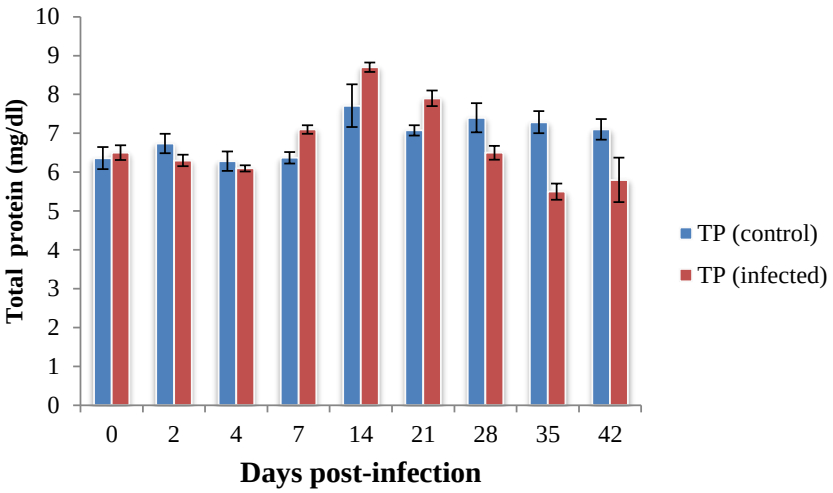

Figure 5. Mean (± SEM) total protein concentrations of *P. multocida* experimentally-infected and control groups in layers.

on days 7 to 28 pi in the *P. multocida*-infected layers compared to the control strongly suggests renal function impairment which may be due to the effect of *P. multocida* and/its endotoxin on the renal tubules or could be as a result of dehydration due to diarrhoea which is in line with the report of Harris (2000). In the same vein, the significant increase in the urea concentration in this study could be as a result of increased catabolism of tissue proteins in other to release energy consequent to anorexia that was earlier reported. This finding was similarly reported by Campbell (1998). The microscopic examination of the kidney in the *P. multocida*-infected layers showed degenerative and necrotic lesions of the renal tubules and which might have affected the renal excretion of uric acid which similarly reported by Harrison and Harrison (1986) and Amany (1997).

Total protein concentration is an important indicator of health status and production features of any organism because of its numerous roles in the physiology and diagnostic purposes (Geogieva et al., 2009). The initial significant increase ($P < 0.05$) in the mean total protein concentration in the *P. multocida*-infected group observed on days 7, 14 and 21 pi could be due to haemo-concentration that might have resulted from diarrhoea, while the subsequent decrease in the mean total plasma protein concentration on days 28, 35 and 42 pi following this rise may be due to malnutrition, that resulted from the infection-induced anorexia (Petersen et al., 2001), or could be as a result of combined effects of reduced hepatic synthesis or impaired renal reabsorption and consequent loss of protein in urine and diarrhoea which agrees with the findings by Campbell and Cole (1986) and Samia (2009).

Conclusion

In conclusion, experimental infection significant increase in plasma concentrations of AST, ALT, ALP, uric acid, and decrease in total protein thus, signified hepatic, intestinal and renal dysfunctions, due to cytopathiec effect caused by the infecting organism and/or its endotoxin.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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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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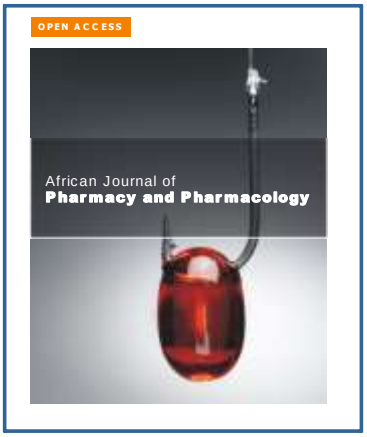

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