World Journal of Pharmaceutical Science and Research
www.wjpsronline.com
Research Article
ISSN: 2583-6579
SJIF Impact Factor: 3.454
Year - 2024
Volume: 3; Issue: 1
Page: 01-17

EFFECT OF *CANARIUM SCHWEINFURTHII* LEAF AND PULP EXTRACTS ON HEMATOLOGICAL, BLOOD CHEMISTRY AND ITS SAFETY IN LABORATORY ANIMAL MODELS

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Article Received: 02 October 2023 | | ***Article Revised: 22 October 2023*** | | ***Article Accepted: 29 November 2023***

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ABSTRACT

Background: *C. schweinfurthii* is a common medicinal plant used as food and medicine in communities of central Uganda. Local communities and herbalists commonly used it in management of aneamia with limited information on its effectiveness on hematological, blood chemistry and its safety in Wistar albino rats. **Aim:** To assess the effect of *C. schweinfurthii* aqueous and total crude leaf and pulp extracts on hematological, blood chemistry and safety in Wistar albino rats. **Methods:** An experimental laboratory based study was conducted on 18 groups each with 6 animals. Active dose of 1000mg/ml aqueous pulp was used for sub-acute toxicity since no death occurred at limit dose of 5000mg/kg bwt. Hematological parameters were measured using an automated Beckman coulter coulter A-T Pierce hematology analyzer. Blood chemistry was analyzed using COBAS INTEGRA 400 Plus. Histological changes in liver and kidney after 28 days of daily dosing of extracts was established. Study was approved by relevant IRB. **Results:** No death was observed at limit dose of 5000mg/kg bwt within 24 hours after dosing indicating its safety. Total bilirubin (µmol/L) was significantly reduced by the extract on day 14 ($p < 0.05$) while direct bilirubin was increased on day 14 and 21. Total protein (g/L) was significantly reduced on 7, 14 and 28 but increased on day 21($p < 0.05$). There were no histopathological changes to the liver and kidney after 28 days of dosing extract. **Conclusions:** Extract was safe at limit dose of 5000mg/kg bwt in animals. No liver and kidney organ toxicity was observed. The plant is safe for use as food and medicine and hence its continued use by the local communities.

KEYWORDS: *Canarium Schweinfurthii, hematological, blood chemistry, safety, histopathological changes*.

INTRODUCTION

Anaemia, one of the most common and widespread dis-orders in the world, is a public health problem in both industrialised and non-industrialised countries. (McLean, Cogswell, Egli, Wojdyla, & De Benoist, 2009). The highest

prevalence of anemia exists in the developing world where its causes are multi-factorial. In the developing world, 42% of children less than five years of age and 53% of children 5–14 years of age are anemic. Anemia has been related to reduced work capacity, reduced ability to execute activities of daily living, poor pregnancy outcomes, and reduced cognitive function. With limited resources and the complex, often multi-factorial nature of anemia in the developing world, combating this problem is a global public health challenge (Rakanita et al., 2020; Tolentino & Friedman, 2007) According to the World Health Organization (WHO), anemia is a condition defined as hemoglobin (Hb) levels <12.0 g/dL in women and <13.0 g/dL in men (Beutler & Waalen, 2006). The number of red blood cells or their oxygen-carrying capacity is insufficient to meet physiologic needs, which vary by age, sex, altitude, smoking, and pregnancy status (Alsayegh et al., 2017).

The World Health Organization (WHO), estimates that over 2 billion people are anemic globally with more than 100million of these cases being anemic children living in Africa (Stevens et al., 2013). Anemia affects over 50% of pre-school children and pregnant women in developing countries and at least 30-40% of these occur in the developed countries. Anemia presents in three clinical forms including mild when Hb levels is between 10 and 11 g/dL, moderate form when Hb between 7.0 and 9.9g/dL and severe form where Hb levels below 7.0g/dL (Mahamoud et al., 2020). The WHO criteria for anemia in children aged 24-59 months is 11g/dL Therefore, because of these challenges encountered by local communities, in the management of anemia, they seek for alternative form of treatments especially involving medicinal plants. (Awodele & Osuolale, 2015). (Organization, Ageing, & Unit, 2008). On the other hand, anemia is also on the rise.

The access to modern Conventional drugs commonly used in the management of anemia such as hematinic agents pose a problem due to high cost, to ordinary people in communities. Their inaccessibility and associated adverse drug reactions (ADR) (Kakudidi, Kirimuhuzya, Anywar, Katuura, & Kiguli, 2016), cost, poor services at healthcare facilities, chronicity of the disease, due to poverty leading to poor nutrition, Therefore, because of these challenges encountered by local communities, in the management of anemia, they seek for alternative form of treatments especially involving medicinal plants that are believed to be free from side effects, cheap and are easily accessible to meet their health needs. Among the commonly used medicinal plants in Uganda, in the management of anemia is *Canarium schweinfurthii (C. schweinfurthii).* However, its effects on hematological parameters and its safety have not been scientifically evaluated despite of its wide use. An experimental laboratory-based study evaluated the activity of *C. schweinfurthii* aqueous and total crude leaf and pulp extracts on hematological, blood chemistry and its safety in Wistar albino rats.

MATERIALS AND METHODS

Study design

An experimental laboratory-based study which used *Canarium schweinfurthii* aqueous and total crude leaf and pulp extracts that were given orally in single daily dose to Wistar albino rats for 28 days. This study was undertaken to investigate the effects of *C. schweinfurthii* medicinal plant on hematological, blood chemistry parameters and its safety in Wistar albino rats.

Study setting

The experiments were conducted at the Department of Pharmacology and Therapeutics laboratory and histopathology was done at the Pathology department at Makerere University College of Health Sciences (MakCHS) in Kampala, Uganda.

Canarium schweinfurthii selection, collections and authentication

C. schweinfurthii leaves and fruit pulps were harvested on 10th September 2021from a farmland at Magere which is located along Gayaza Road, north of Kampala city Centre in Wakiso district in Central Region of Uganda. The plant was identified by a taxonomist at Makerere University who gave it a voucher number 50920 and was deposited at Makerere University herbarium for future reference. The leaves and fruit pulp were sorted, damaged and rotten ones were discarded (Olawale, 2012). The fruit pulp was removed from the seed before drying. The leaves and fruit pulp of *C. schweinfurthii* samples were air-dried separately at room temperature under a shade at the Department of Pharmacology and Therapeutics laboratory until a constant weight was attained. Each dry sample was pounded separately to coarse powder using motor and pestle, to ease the extraction of active compounds. The powder was kept in an air tight container in preparation for the extraction process.

Extraction process

The process followed the already established extraction procedure of plant samples, (Ciulei, 1964; Cowan, 1999).. Serial extraction methods in which the powder was first soaked in diethely ether; then followed by methanol and lastly water as solvents were used. Three hundred (300 g) plant powder was soaked in 700 ml diethyl ether (98%) in Ehlnmeyer flaskf for 3 days. Three hundred (300g) of each of the leaf and pulp powder were weighed using an electronic weighing scale (Mettler PJ3000, Mettler-Toledo GmbH, Ockerweg, Germany), and cold macerated in 1.6 liters of absolute diethyl ether (Zayo-Sigma, Germany) in amber colored bottle for 3 days being shaken for 2 hourly for 12 hours. The mixture was filtered after 72 hours, using a gauze cloth, and the fine filtrate was obtained using the Whitman No: 1 filter paper in Buchner funnel. Rotary evaporator (BUCHI Rotavapor R-205) was used to retrieve the diethyl-ether and methanol solvents. The dry residue was air dried at room temperature for 3 days.

The same process was repeated on the residue using methanol (95% V/V) for 3 days.

The dried residue was soaked in 1.3 L distilled water at 96°C to avert fungal attack and cooled at room temperature. The mixture was shaken 2 hourly to facilitate extraction for 12 hours. The filtrate was freeze dried at a pressure of 32 Pa, original temperature was set at -47°C and then maintained at 0°C for 36 hours to dry the extract. The powder of diethyl ether, methanol and aqueous extracts were weighed, and 1L of distilled water added to make a stoke solution from which the daily doses were calculated according to rats body weight and the allocated daily dose.

Preparation of stock solutions

The 4g of each extract were added a few drops of dimethyl sulfoxide (DMSO) and then topped up to 4mls to produce 1000mg/mL. Then serial dilution of each of the extract solutions was made to produce varying doses (1000, 500,250,100mg/mL) that were used in the experimental studies.

Preparation and treatment of experimental animals

One hundred and eight (108) Wistar albino rats aged 6-8 weeks; adult disease-free rats were used for the study. They were bred from Makerere University College of Veterinary Medicine, Animal resources and Biosecurity, animal house from where they were transferred to the Pharmacology laboratory at the Department of Pharmacology and Therapeutics in preparation for the experimental study. The animals were accommodated in the cages at the department for a week to acclimatize to the new environment before commencement of the study. The experimental animals were kept at standard laboratory conditions of temperature (25±1°C), relative humidity (45-55%) and light/dark cycle (12hr light: 12hr dark cycle). Standard rat pellets (food) and clean water were provided *ad libitum* to the animals (Gordon, 2001a). This was done according to the National academies press guideline for care and use of laboratory animals (Council, 2010). The 3Rs: Replacement, Reduction and Refinement were observed based on international standard guidelines on use of laboratory animals in biomedical research. The 5 freedoms of animal welfare which are globally, recognized as the gold standard in animal welfare, encompassing both the mental and physical well-being of animals were observed including: freedom from hunger and thirst; freedom from discomfort; freedom from pain, injury, and disease; freedom to express normal and natural behavior (Sneddon, Halsey, & Bury, 2017).

Selection of experimental animals

Inclusion criteria

Adult Wistar albino rats at the age 6-8 weeks, which were judged to be healthy by their record of good appetite and clear solid stools. Their body temperature was measured using a body thermometer placed in the rectum to ascertain that they are not febrile. Temperature of 37°C was considered normal.

Exclusion criteria

Any rats that showed signs of ill-health like failure to feed; having diarrhea, a runny nose or being febrile were not used in the study. Pregnant, nursing rats or those with external wounds and a rough coat depictive of disease were also excluded from study and were treated promptly by the qualified veterinary doctor.

Sample size estimation

Wistar albino rats aged 6-8 weeks, which were randomized into 18 experimental groups (n=108) with six adult rats in each group.

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Wistar albino rats aged 6-8 weeks, which were randomized into 18 experimental groups (n=108) with six adult rats in each group.

Group treatment and dosing of animals

The study comprised of one hundred and eight (108) (Table 1) based on the Organization of Economic Co-operation and Development (OECD) guidelines 407, that recommend that a minimum of 6-10 animals per group be used, to which can be added the number of animals scheduled to be euthanized during the study period (Gordon, 2001b) (Council, 2010).

Determination of safety of *Canarium schweinfurthii*

Acute toxicity LD 50

Adult mice 6-8 weeks were used to determine the percentage death of animals within 24 hours after an oral single extract dose administration. Aqueous leaf and pulp extracts were given orally (through an intragastric tube) in single doses to 6 mice (test groups), after being starved for 6 hours. The animals were divided into a control group of 2 animals, and four treatment groups that were dosed with different upgraded doses of each extract (2000, 3000, 4000 and up to a limit dose dose of 5000 mg/kg of extract) to each mouse in all group. The oral toxicity study was carried out according to OECD guideline 407 (Burger, Fischer, Cordenunzzi, Batschauer Filho, & VC, 2005). Death, behavioral and clinical signs and symptoms were observed continuously for the first 4 hours after dosing, then hourly for the next 24 hours, and finally, 6-hourly for 72 hours (Ayub, Garg, & Garg, 1997).The mortality of the animals were expressed as leathal dose at 50 (LD50), a dose that kill 50% of the test animals following administration of the animals. However, there was no death recordered even after the 5000mg/kg bwt limit dose. Therefore the highest test dose used of (1000mg/kg bwt) was used for sub-acute toxicity study.

Determination of Sub-acute toxicity

This study was undertaken to determine the effects of daily dosing of the 1000mg/kg bwt of the extracts for 28 days on hematological parameters, serum enzymes and organ toxicity (liver and kidney). The study used 8 to 10 weeks old wistar albino rats that were bred from Makerere University College of Veterinary Medicine, Animal resources and Biosecurity animal house. The animals were subjected to normal 12 hours day light and 12 hours of night darkness. They were fed on commercial rat pellets from Engaano millers and had access with distilled water ad libitum. Two (2) groups comprising of 6 rats each were used (6 animals in control group and 6 animals in test group). Since there was no death for the acute toxicity study, the most active dose of the extract (aqueous pulp at a dose of 1000mg/kg body weight) was used. Blood samples were collected at day 0, 7, 14, 21 and 28th day using gauge 23 needle from tail vein into a purple top vacutainer with EDTA as anticoagulant. Blood samples (1.0ml) were divided in two portions with 0.5 ml put in a (purple top) for hematological parameters analysis and the other 0.5mL in a red top vacutainer for serum extraction which were used for clinical chemistry (RFTs and LFTs) analysis. Hematological parameters were measured using an automated Beckman coulter coulter A-T Pierce hematology analyzer (Beckman, Coulter, inc. Fullerton, CA, USA) machine at the department of Medical Physiology laboratory based on the manufacturer standard methods and procedures. Blood chemistry (alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, creatinine phosphokinase, gamma-glutamyl transpeptidase, lactate dehydrogenase, total proteins, blood urea, bilirubin and creatinine) were analyzed using COBAS INTEGRA 400 Plus clinical chemistry analyzer at the department of Medical Physiology laboratory based on the manufacturer standard methods and procedures. Then on the 29th day, the animals were anesthetized with 20mg/kg bwt Phenobarbital and then sacrificed. Immediately the kidney and liver organs were harvested and preserved in 10% formalin. The tissue slides were made, stained with haematoxylin and Eosin at the department of Pathology, Makerere University College of Health Sciences and were examined under light microscope of magnification x10 by a Pathologist for any histopathological changes due to the administered extract dose.

Quality control and data management

A well trained and experienced research assistant in handling of laboratory animals were involved in the preparation of the animals and carrying out the procedures. All chemicals and reagents used were of analytical grade and were checked to ensure that they were not expired before the experimentation. Sample analysis were replicated in order to

ensure that they were valid and reliable. Equipments used were using recommended methods and procedures and all the time they were calibrated. Using inbred rats of similar age group and were in the same environment, same diet to reduce differences in metabolism. Healthy animals were used throughtout the experimental studies.The data collected was entered in Microsoft Excel 2013 spread sheet from where they were exported into STATA version 13 for statistical data analysis.

Data analysis

Data was analyzed using STATA version 13 and the means and standard error for mean (SEM) for each treatment group was obtained. Comparisons of means were used to determine statistical significance of the results using one way ANOVA followed by a boniferroni test for binary comparison of the treatment groups. The results were presented in form of tables, graphs and figures.

Ethical considerations

Permission to conduct the study was sought from the Department of Medical Physiology, the School of Biomedical Sciences Institutional Review Board (IRB) and the Uganda National Council for Science and Technology. The protocol was approved and numbered SBS-812. Ethical practices that govern handling of laboratory animals were adhered to as per international biosafety guidelines and the guidelines for the care and use of laboratory animals (Gordon, 2001a). Training in animal care and the use of animals for research was done on line with “The Global Health Network” and a certificate Number ca258ea3-580f-4d4c-846c-30305c569442 Version number 0 was issued. The approved protocol was also reviewed and approved by the Institutional Animal Care and Use Committee at COVAB and given a reference #SVAR-IACUC/66/2020.

RESULTS

Effects of 1000mg/kg bwt aqueous pulp extract dose administration on hematological parameters in Wistar albino rats

The effects of the daily dosing of the extract on hematological parameters complete blood count, white blood cell count (WBC), red blood cell count (RBC), differential counts (neutrophils, eosinophils, monocytes, basophils and lymphocytes) were noted (figure 5).

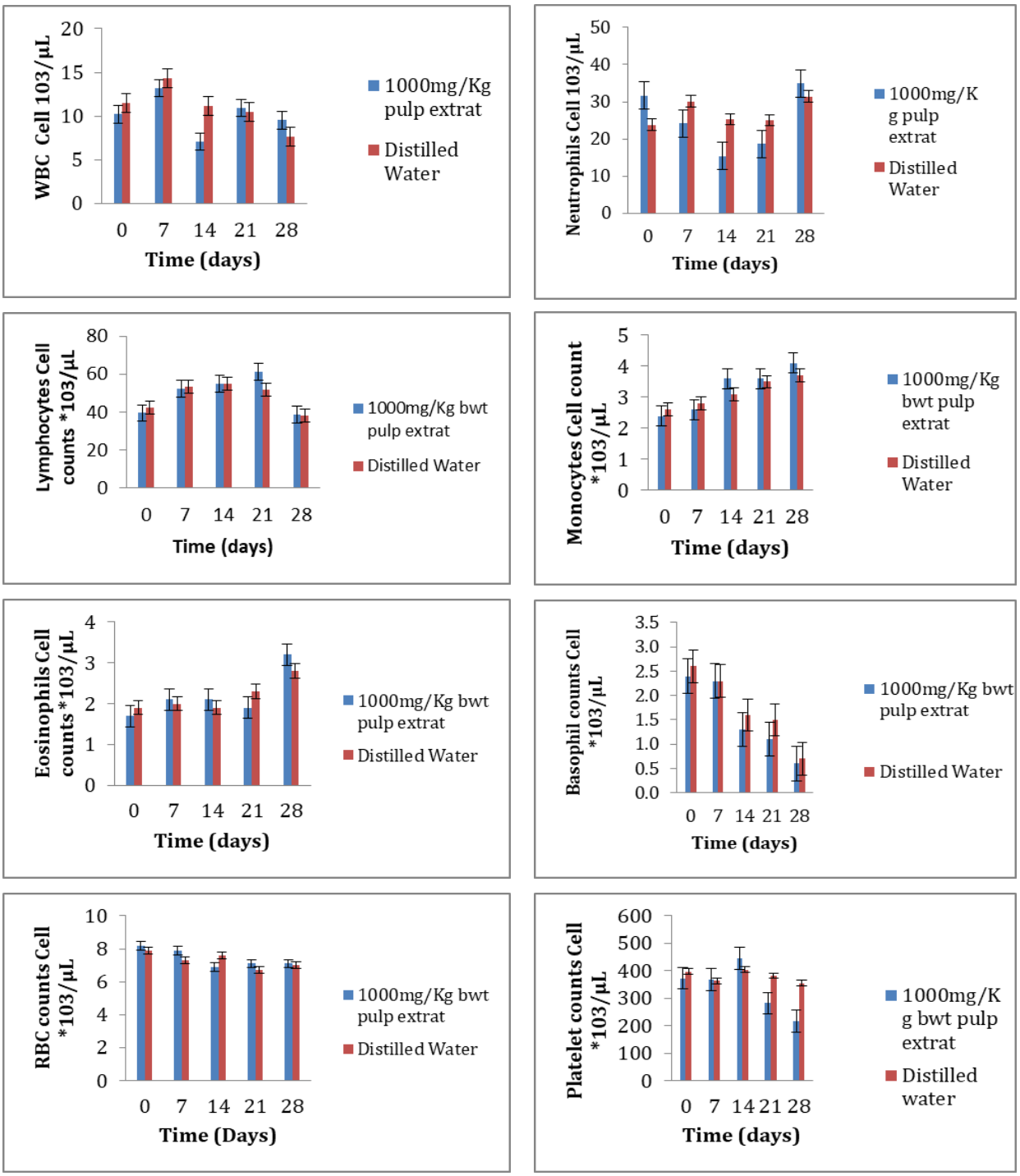


Figure 2: Effects of 1000mg/kg bwt aqueous pulp extract dose administration on hematological parameters in Wistar albino rats.

The findings showed that hematological parameters of the Wistar albino rats treated with *C. schweinfurthii* remained within the normal range during the duration of the experiment. The mean white blood cell count (WBC) was significantly reduced on day 14 and increased on day 28 as compared to the distilled water control group; mean red blood cell count (RBC) were slightly increased on day 14, 21 and 28; the mean neutrophil count were significantly reduced by the extract dosing at day 14 and 21 and then increased on day 28; mean lymphocytes count was increased on day 21; mean monocyte count was increased on day 14, 21 and day 28; mean eosinophil count was increased on day

7, 14 and 28; mean basophil count was reduced on day 14, 21 and day 28 while the mean platelet count were increased on day 14 and then significantly reduced on day 21 and 28 (figure 1)

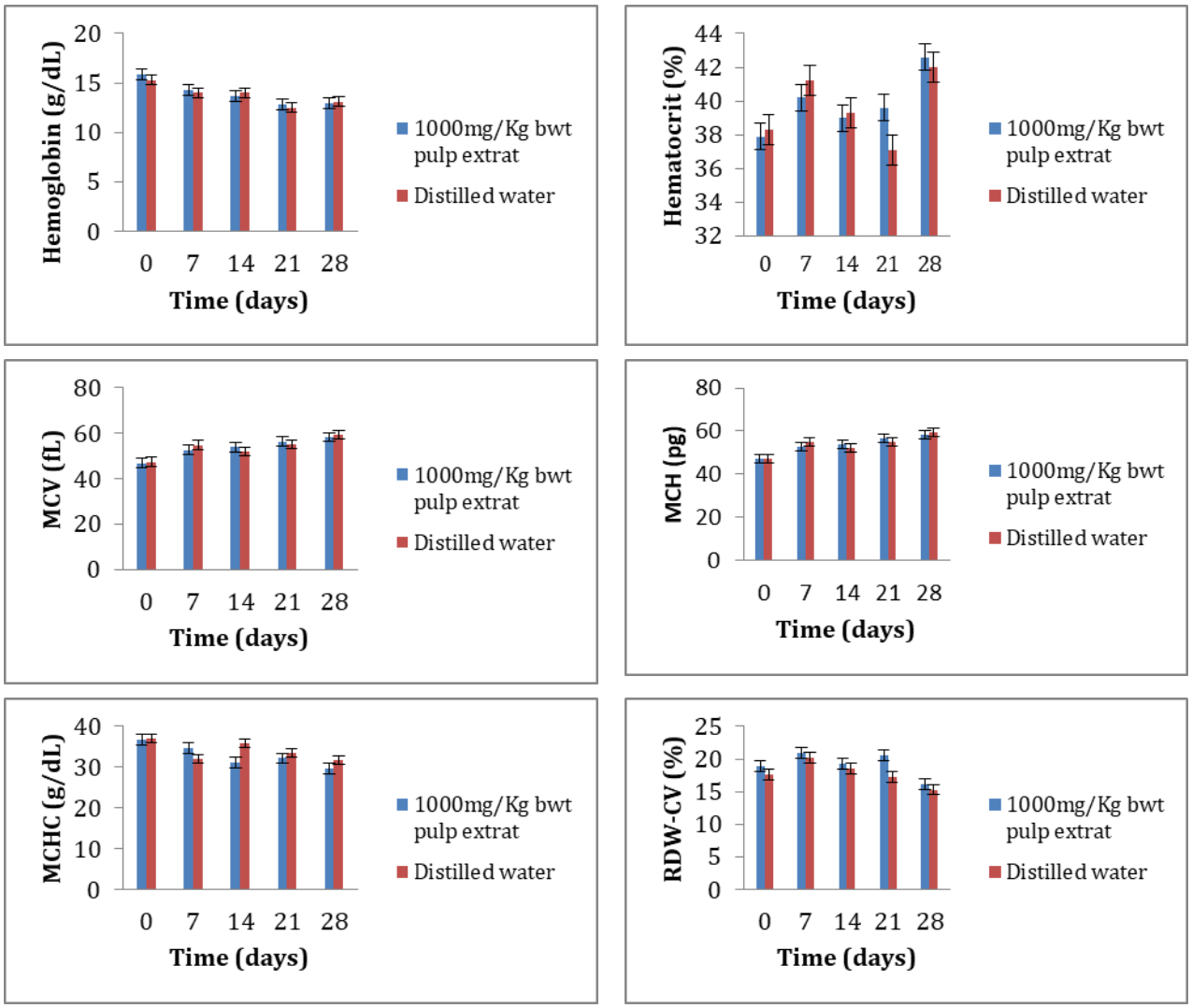


Figure 3: Effects of 1000mg/kg bwt dose aqueous pulp extract administration on RBC indices in Wistar albino rats.

The effects of the daily dosing of the extract for 28 days on RBC indices, (hemoglobin, hematocrit, MCV, MCH, MCHC and RDW-CV) were investigated. The effects of the extract dosing on RBC indices, the findings show there was no difference on hemoglobin levels on day 0, 7, 14, 21and 28 to that of distilled water (figure 3). The effect of the extract hematocit, the finding show that the % hematocrit, the finding show that the % hematocrit was slightly lower to that of distilled water on day 7 and 14 and significantly higher on day 21. However, there was no difference on the effects of extract dosing on MCV and MCH levels on day 0,7,14 ,21, and 28 to that of distilled water (figure 3). The effect of the extract on MCHC levels were slightly lower to that of distilled water on day 14,21 and 28 following dosing. Though, the MCHC levels were significantly lower ($P< 0.05$) to that of distilled water on day 14 of dosing. Finally, the effects of the extract on % RDW/CV, the findings show that, there was an increase in the % on day 14, 21 and 28 and the levels were significantly higher ($P \leq 0.05$) to that of distilled water on day 21 of dosing (figure 3).

Effects of 1000mg/ kg bwt aqueous pulp extract dose administration on the blood chemistry levels in Wistar albino rats

The effects of the daily dosing of the aqueous pulp extract for 28 days on blood chemistry (total bilirubin, direct bilirubin, ALT.AST, ALP, CREA, Urea and total protein) were investigated (figure 3).

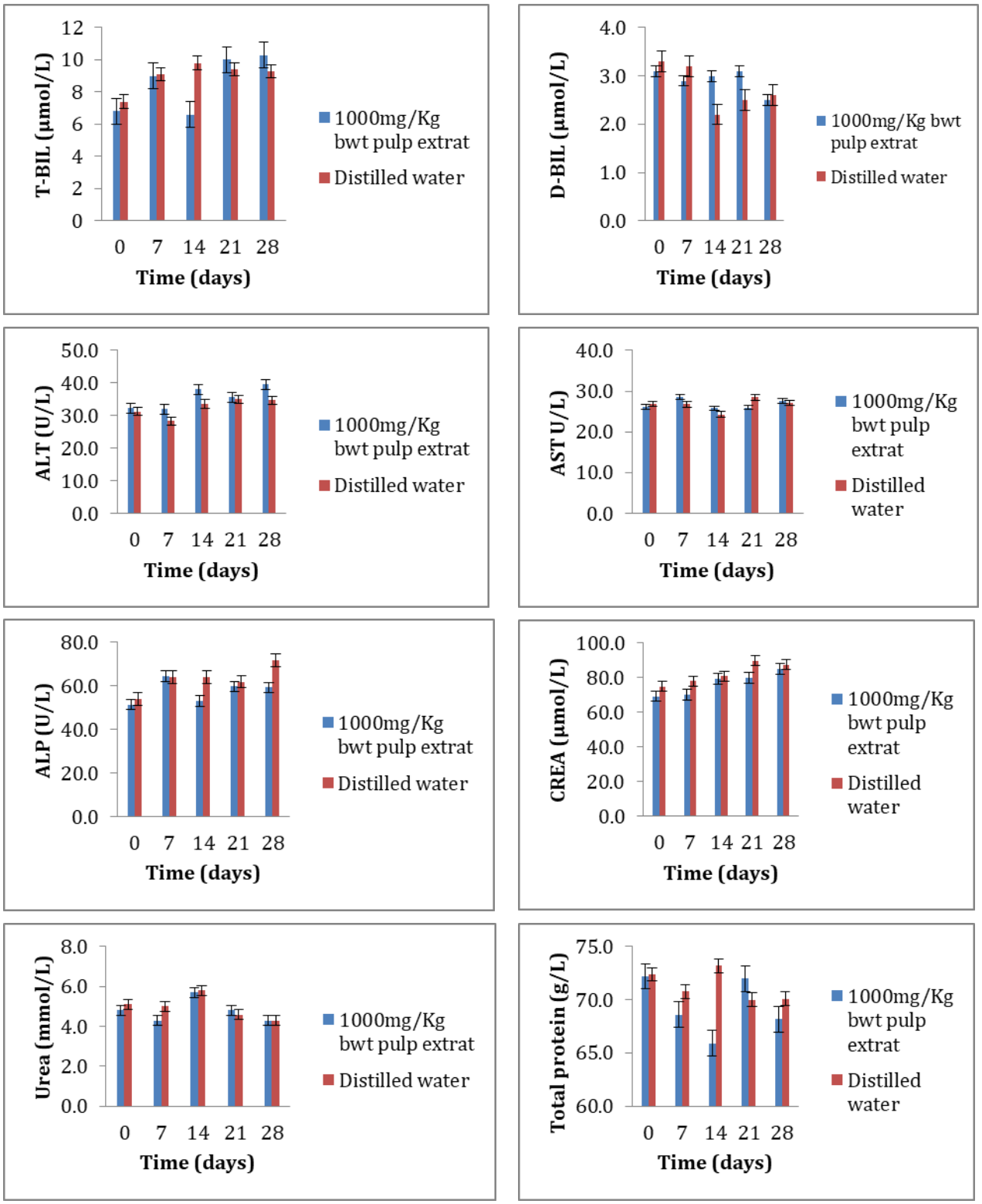


Figure 4: Effects of 1000mg/ kg bwt aqueous pulp extract dose administration on the blood chemistry levels in Wistar albino rats.

The effects of the daily dosing of the aqueous pulp extract for 28 days on blood chemistry (total bilirubin, direct bilirubin, ALT.AST, ALP, CREA, Urea and total protein) and the findings showed that their serum levels remained normal in the treated groups and control groups throughout the course of treatment.

Effect of the extract on total bilirubin levels

The finding shows that the total bilirubin (T-bil) levels of the test group were almost similar to the distilled water (control group) at day 0,7,21, and 28 days of dosing. However, on the day 14 the total bilirubin levels were significantly lower than that of the distilled water (figure 4). The effects of the extract on the direct bilirubin levels (D-bil) similar to normal saline on day 0,7and 28 but it was significantly higher ($P \leq 0.05$) % that of distilled water at day 14 and 21. The effects of the extract on alanine aminotransferase (ALT) levels ,the findings show the levels of ALT were slightly higher than that of distilled water at day 7, 14 and 28. The effect of the extract on aspartate aminotransferase (ASTA) levels, the findings show that levels were slightly higher than that of distilled water at day 7 and 14 and then lower than that distilled water at day 21.

The effects of the extract alkaline phosphatase (ALP) level, the finding show that the ALP was significantly ($p < 0.05$) lower than that of distilled water at day 14 and 21. The effects of the extract on creatinine (CREA) levels, the findings show that the levels were slightly lower than that of the normal saline at day 7and 21. The effects of the extract dosing on the urea levels, the findings show that levels, were lower than that distilled water at day 7 and there was no difference on day 0, 14, 21 and 28. Finally, the effects of the extract on total protein levels, the findings show that the levels were significantly lower ($P < 0.05$) to that of normal saline on day 7,14 and 28 and significantly higher ($P < 0.05$) at day 21 (figure 3).

Findings of acute toxicity study

No animal died at maximum limit dose of 5000mg/kg within 24 hrs in all treated groups.

Effects of 1000mg/kg bwt dose aqueous pulp extract administration on major body organs (Liver and Kidneys) in Wistar albino rats

The histopathological examination of liver and kidneys from rats which received 1000mg/kg bwt daily oral dose of *C. schweinfurthii* for 28 days (Figure 5). The findings showed that the hepatocytes and kidney architecture remained normal. Both the controls and rats that received the intervention showed no signs of inflammation there was no sign of toxicity (Figure 5).

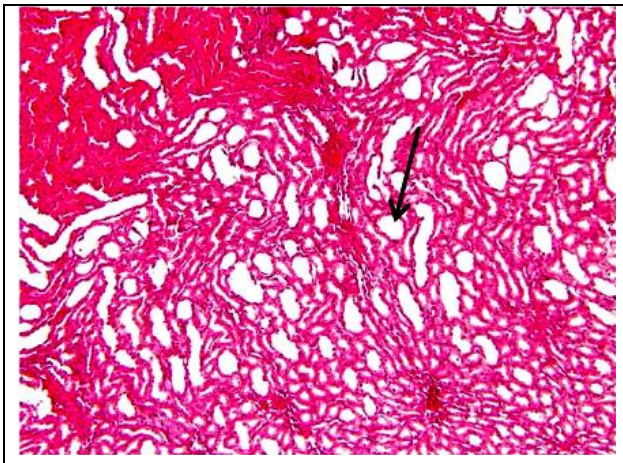
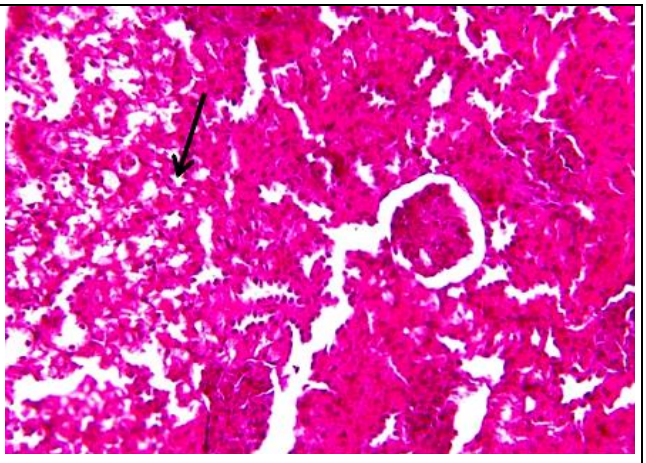
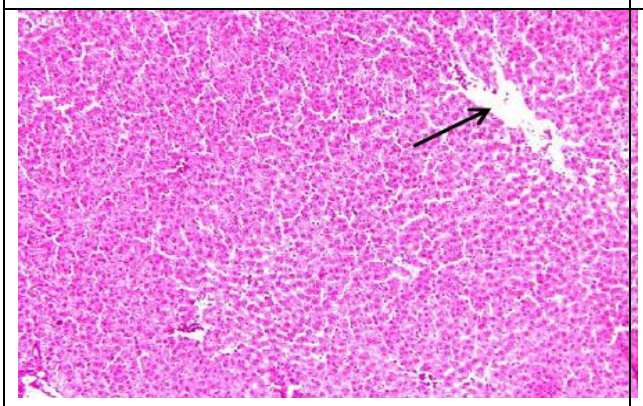
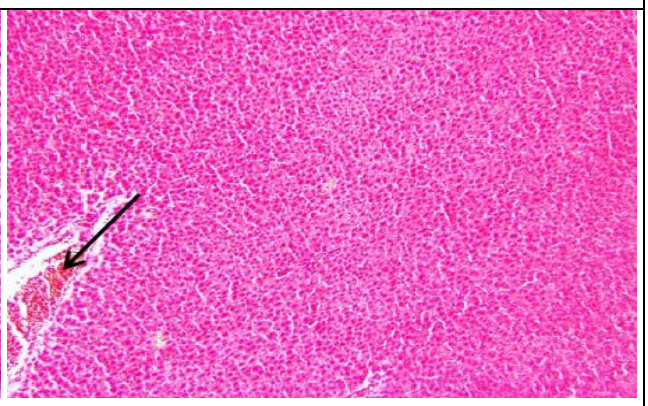
	
Photomicrograph 1: Arrow shows a section of the kidney, showing normal collection tubules in a control with Distilled water (x10 magnification. Hematoxylin & Eosin stained.	**Photomicrograph 2: Arrow shows a section of the kidney showing normal collecting tubules in a case effects of 1000mg/kg bwt C. Schweinfurthii aqueous pulp extract administration for 28days effects on the kidney in Wistar albino rats (X10 magnification Hematoxylin & Eosin stained.**
	
Photomicrograph 3. Section of the liver showing normal architecture, hepatocytes and central venule in a control (BA Control) with Distrilled water (X10 magnification, hematoxylin & Eosin stained)	**Photomicrograph 4. Section of the liver showing architecture, hepatocytes and central venule in a case (GT) effects of 1000mg/kg bwt after aqueous pulp extract of Canarium Schweinfurthii administration for 28 days on effects on the liver in albino rats (x10 magnification, Hematoxylin & Eosin stained)**

Figure 5 : Histopathological effects of daily dosing of the 1000mg/kg bwt extract for 28days on the kidney and liver of Wistar albino rats.

DISCUSSION

Percentage yield

The yield from aqueous extract was 2.5% while that from the methanol was 3.7%. The aqueous extract provided less yields than the methanol because methanol extracts is stronger with more phytochemicals.

Effects of 1000mg/kg bwt total crude extract dose administration on hematological parameters in Wistar albino rats

The effects of the daily dosing of the extract on hematological parameters complete blood count, white blood cell count (WBC), red blood cell count (RBC), differential counts (neutrophils, eosinophils, monocytes, basophils and lymphocytes) were investigated. The analysis of hematological parameters, which included WBC, RBC, GRA, HB, (Fig 2) in rats treated with *C. Schweinfurthii* aqueous pulp extract (1000 mg/kg bwt) did not differ significantly from those of control rats. (Figure 2). Blood parameters reflect the healthy state of an organism. This finding is in close agreement with a study done in Cameroon (Kharchoufa et al., 2020), on Hematological Parameters did not differ significantly from those of control rats. They did not have any marked effect on white and red blood cells counts, hemoglobin content and hematocrit percentage. Previous studies have shown that hematological parameters were very sensitive and could be used as reliable indicators for determining the intrusion of toxic substances (Rahman, Siddiqui, & Jamil, 2001).

The implication of this study is that the administration the aqueous pulp extract of *C.Schweinfurthii* probably do not affect the hematological parameters of the experimental animals, therefore can be used by humans.

Effects of 1000mg/kg bwt dose aqueous pulp extract administration on RBC indices in Wistar albino rats

The changes in the hematological indices for the rats in both extract groups were not significant. However, the red blood cells parameters such as Hb, MCHC, MCH, PCV, MCV and RCDW-CV (Figure 3) were studied to investigate the beneficial effect of *C. Schweinfurthii* pulp extract on the anemic status of the diabetic rats. Following plant extract administration, the level of RBC and its related indices remained almost no difference to the controls. This gives an indication that the plant extract may contain some phytochemicals that can stimulate the formation or secretion of erythropoietin in the stem cells of the animals. Erythropoietin is a glycoprotein hormone which stimulates stem cells in the bone marrow to produce red blood cells. The stimulation of this hormone enhances rapid synthesis of RBC which is supported by the improved level of MCH and MCHC (Abu-Zaiton, 2010).

The effect of 1000mg/kg bwt aqueous pulp extract administrations on blood chemistry in Wistar albino rats

Hepatic and renal function are crucial, with one being used for the metabolism of ingestion and the other for excretion of the waste product, respectively (Kasolo, Bimenya, Ojok, & Ogwal-Okeng, 2012). To evaluate the toxicity of any new compound, it is essential to know the state of these two vital organs, which can be verified by biochemical estimation. In this study, liver function and renal function tests were in normal range for all treated rats. Protein profile and metabolic biomarkers were also remained normal. Serum levels of three enzymes (ALT, AST, and ALP) are commonly used as clinical biochemistry markers associated with liver damage. Among these enzymes, serum levels of ALT, ALP and AST (Figure 4) of the *C.Schweinfurthii* 1000, mg/kg/bwt pulp extract were comparable when compared to the control. The study showed no biochemical alterations in the liver after assessing serum liver enzymes (ALT, AST, and ALP) (Figure 4). Liver function tests help in the diagnosis of any abnormal/normal condition of liver. Hepatic cells are involved in a variety of metabolic events; therefore, the intervention with aqueous pulp of *C.Schweinfurthii* during the experiment, would have indicated any abnormalities that could have been inflicted to the liver. The transaminase enzymes; Alanine transaminase (ALT), Aspartate transaminase (AST) and Alkaline phosphatase (ALP) are the most employed indicators of hepatocellular damage (Adeniyi et al., 2016).The elevated liver enzymes may indicate inflammation or damage to cells in the liver. The inflamed or injured liver cells leak higher than

normal amounts of certain chemicals, including liver enzymes, into the bloodstream, which can result in elevated liver enzymes on blood tests (Fakurazi, Sharifudin, & Arulselvan, 2012).

Moreover, ALT and AST levels also act as indicators of liver function and maintenance of normal levels of these parameters indicates normal functioning of the liver (Kouambou et al., 2007). The transaminases values in the treated group were not statistically different ($P > 0.05$) compared to the control group. This attested that the 1000 mg/kg bwt dose of *C. Schweinfurthii* plant extract did not produce adverse effect on the liver function. This is in accordance with the non-significant difference ($P > 0.05$) in the same parameters observed by Coulibaly et al. (2018). This study found that there was no increase in the levels of all liver injury biomarkers. Bilirubin is a byproduct of red blood cell lysis and serves as a marker of hepatocellular function. Bilirubin is derived from the heme molecule, which is abundant in red blood cells. Bilirubin is released to the circulation with red blood cell lysis and is cleared by hepatocytes. Once inside the hepatocyte, bilirubin is conjugated (to glucuronide, primarily) and exported to bile for excretion (Jamkhande, Barde, Patwekar, & Tidke, 2013).The mean values for D-dilurubin and total bilirubin of rats after 28 days of intervention have been shown in Figures(4 AST U/L and 4 ALP U/L) . No significant effects have been observed in the levels of bilirubin of rats treated with 1000mg/kg bwt, of extract *C. schweinfurthii* as a single daily dose for 28 days. In the present work, there was no significant change in serum creatinine, Urea both in control and treated diabetic rats (Kouambou et al., 2007). Creatinine is a non-protein nitrogenous metabolite that is cleared from the body by the kidney, following glomerular filtration (Nwankpa et al., 2018) The estimation of the level of this metabolite is employed as marker for kidney function (Assessment of creatinine level showed that there was no significant change in values ($P > 0.05$), in the animals treated group compared to the control group. This was not in agreement another study done in Nigeria which showed no difference in creatinine values observed in the treated group ((Okwuosa, Achukwu, Nwachukwu, Eze, & Azubuike, 2009)).Toxicity profiles shows no mortality and harmful effect in rats using single dose of 1000 mg/kg bwt. of Canarium Schweinfurthii exposed group and control groups (Figure 3). The liver has a significant role in plasma protein synthesis, notably it is the site for the synthesis of practically all albumin and fibrinogen and probably more than 80 per cent of the globulin fractions (Miller, Bly, Watson, & Bale, 1951). After 14 day the total protein slightly decreases probably due to destruction which was later increased a week later on day 21. The findings from this study suggest that probably *C. Schweinfurthii* aqueous pulp has no short-term effect on the synthetic function of the liver.

Effects of 1000mg/kg bwt dose aqueous pulp extract administration on major body organs (Liver and Kidneys) in Wistar albino rats

Histological assessment of liver, kidneys showed no variation in liver neither kidneys architecture in rats treated sub acutely with *C. schweinfurthii* extract. On the other hand, no changes were observed in biochemical markers of liver and renal function in all rats treated. In light of this similarity between the biochemical and histological results of the liver and kidneys it can be noted that *C.schweinfurthii extract* probably does not present nephrotoxicity alteration of the functions of the liver and kidneys. However, a chronic study is needed for the complete understanding of the liver toxicity and nephrotoxicity of this plant. The present study was the first one of its kind that evaluated the short-term subacute toxicity of Aqueous *C. schweinfurthii* pulp extract. We aimed to assess the possible toxicity effects of *C. schweinfurthii* aqueous pulp extracts on major vital organs, such as kidneys and liver which have more important roles in the detoxification of exogenous chemical substances than other organs. The current study had a 28-day duration of typical prechronic toxicity studies recommended by regulatory guidelines (Rothfuss et al., 2010). Therefore, it should

be taken into account that longer exposure periods (90 days for example) may also yield different results (Kharchoufa et al., 2020). The study implication is probably *C. schweinfurthii* aqueous pulp extracts on major vital organs, such as kidneys and liver does not cause toxicity.

CONCLUSION AND RECOMMENDATION

The yield from aqueous extract was 2.5% while that from methanol was 3.7%. The aqueous extract provided less yields than the methanol because methanol is an ideal solvent that extracts both hydrophilic and lipophilic molecules from plant plants. *C. schweinfurthii* aqueous leaf and pulp extracts on RBC, HB, MCV and MCHC (hematological indices) in Wistar albino rats were not affected and were within the normal range. *C. schweinfurthii* leaves and pulp aqueous extracts fed to rats orally in a single dose for 28 days was probably not associated with short term liver or kidney toxicity and no changes were observed in biochemical markers of liver and renal function in all rats treated. The histopathology findings are in agreement with the blood chemistry results of the liver and kidneys, it can be noted that *C. schweinfurthii* extracts probably is not associated with short term nephrotoxicity or liver toxicity alteration of the functions of the Liver and kidneys.

ACKNOWLEDGEMENTS

I wish to extend my appreciation to my sponsors for this study Makerere Non-Communicable Disease (MakNCD), Research reported in this publication was supported by the Fogarty International Center of the National Institutes of Health under Award Number D43 TW 011401. The content is solely the responsibility of the authors and does not necessarily represent the official views.

Conflict of Interest

There is no conflict of interest to be declared.

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