

*Full Length Research Paper*

## **Safety and toxicity of aqueous leaf extracts of *Camellia sinensis*, *Parquetina nigrescens* and *Telfairia occidentalis* in mice**

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Received 17 December, 2017; Accepted 11 April, 2018

Plant extracts as potential phytotherapeutic products are supposed to be safe. However, adverse and untoward fatal effects have been reported. Study aimed to evaluate the safety and toxicity of aqueous extracts of *Camellia sinensis*, *Parquetina nigrescens* and *Telfairia occidentalis* leaves. The extracts were subjected to brine shrimp lethality bioassay and toxicities by Lorke's method. Mice were given oral leaves extracts of *C. sinensis* (1000, 2000, 4000 mg/kg and 700, 1400, 2800 mg/kg); *P. nigrescens* (3000, 6000, 12000 mg/kg and 2000, 4000, 8000 mg/kg) and *T. occidentalis* (2500, 5000, 10,000 mg/kg and 1750, 3500, 7000 mg/kg) for acute and Sub-acute toxicity studies respectively. Toxicity was observed for the first 4hrs, then over a period of 24hrs and at least once daily for 14 days extended to 28 days. General behavior, adverse effects and mortality observed and evaluated throughout the experimental period. *Camellia sinensis* (LC<sub>50</sub>=418.6 µg/mL) with least toxicity on the brine shrimps compared to *P. nigrescens* (LC<sub>50</sub>=32.34 µg/mL) and *T. occidentalis* (LC<sub>50</sub>=8.32 µg/mL). LD<sub>50</sub> of the extracts; 2800, 8000 and 7000 mg/kg for *C. sinensis*, *P. nigrescens* and *T. occidentalis* respectively. No death, No changes in body and relative organ weights. However, *C. sinensis* and *T. occidentalis* significantly increased in Haemoglobin (*C. sinensis*: 15.90 ± 0.33 (p < 0.00) and *T. occidentalis*: 14.67 ± 0.22 (p < 0.01)), PCV (*C. sinensis*: 46.20 ± 1.02 and *T. occidentalis*: 44.00 ± 0.71 (p < 0.00)), RBC (*C. sinensis*: 5.55 ± 0.12 and *T. occidentalis*: 5.49 ± 0.12 (p < 0.00)). No histomorphological changes in the vital organs except *P. nigrescens* with mild kidney interstitial fibrosis, mild glomerular hypercellularity and mild liver microhemorrhages. Various doses of the extracts did not cause mortality or serious signs of toxicity in mice.

**Key words:** *Camellia sinensis*, *Parquetina nigrescens*, *Telfairia occidentalis*, acute and sub-acute toxicities, No Observed Adverse Effect Level.

## INTRODUCTION

Medicinal plants provide an alternative strategy in search for new drugs (Balunas and Kinghorn, 2005). There is abundance of plants reputed in traditional medicine that possess protective and therapeutic properties (Farnsworth et al., 1985). Plants are leading compounds for the development of new medicines and also for the treatment and prevention of various human ailments (Farnsworth, 1994). It is likely that plants will continue to be a valuable source of novel molecules which may provide new and improved drugs (Fabricant and Farnsworth, 2001). Although, modern medicine has gradually developed in recent years, traditional medicine still receive high patronage (Ernst, 2000) since herbs and herbal medicines are believed to be effective, cheap and free from side effects (Bent, 2008). Thus, herbal remedies have remained as the basis for the development of new drugs (Koehn and Carter, 2005; Cragg and Newman, 2013).

Toxicity associated with herbal products (Bateman et al., 1998; Ernst, 1998) has alerted many national (Awodele, 2014) and international regulatory authorities (Saad et al., 2006) to develop and implement various sets of guidelines for assessing, monitoring, and preventing the toxicity associated with such herbal products (Chang, 1987). Toxicity tests are most widely used to examine specific adverse events or specific endpoints (Isbrucker et al., 2006) such as cancer, cardiotoxicity and skin/eye irritation, etc. Toxicity testing is also helpful in determining the “No Observed Adverse Effect Level (NOAEL)” dose and is helpful for further clinical trials (Setzer and Kimmel, 2003). Acute, sub-acute and chronic toxicity tests are routine safety tests carried out by pharmaceutical companies in the process of developing new medicines (Chang, 1987). However, in order to assess the toxic nature of compounds, acute oral toxicity is the first step to be carried out (Akhila et al., 2007).

Studies have shown that aqueous extract of *Camellia sinensis*, *Telfairia occidentalis* and *Parquetina nigrescens* possess anti-mutagenic, anti-inflammatory, erythropoietic and effective chemo-preventive potentials against toxic chemicals and carcinogens. However, preliminary animal studies need to be done on these plant extracts- as individual and in the combined form so as to provide scientific justification(s) for their consideration in clinical trials and thereby develop them as new radio-protectors and/or radio-mitigators in cancer radiotherapy. More so, the growing number of herbal medicine users globally (Elsenberg, 1998) and the lack of scientific data on the safety profiles of many herbal products however, have make it necessary to conduct safety studies on herbal

products (Chang, 1987), thus justifying conducting this study.

### *Camellia sinensis*

Green tea is a drink made from the steamed and dried leaves of *Camellia sinensis*, a shrub native to Asia (Costa, 2002). Green tea has been widely consumed in the Far East to promote good health for at least 3,000 years and is the second most consumed beverage in the world, with an estimated 18 to 20 billion cups consumed daily and an estimated average consumption of 1 L/person/day in the United Kingdom (Wu and Wei, 2002). This high rate of consumption may be justified possibly because of its biological properties such as: anti-oxidant (Feng et al., 2001), anti-obesity (Brown et al., 2011) and anti-cancer (Feng et al., 2001; Park et al., 2003; Sakata et al., 2004). The chemical composition of green tea is complex; it contains polyphenols, alkaloids (caffeine, theophylline, and theobromine), amino acids, carbohydrates, proteins, chlorophyll, volatile compounds, fluoride, minerals, trace elements and other undefined compounds. Among these, the polyphenols constitute the most interesting group that exhibit potent anti-oxidant activity *in vitro* and *in vivo* studies (Kondo et al., 2002). Although, tea has been considered a medicine and a health-derived beverage since ancient times, recently it has received a great deal of attention because the polyphenols are strong antioxidants (Feng et al., 2001; Sakata et al., 2004). Numerous studies have also demonstrated that the aqueous extract of the major tea polyphenols possesses anti-mutagenic (Sakata et al., 2004), anti-diabetic, anti-bacterial, anti-inflammatory, and lipid-cholesterol lowering properties (Brown et al., 2011; Olatunbosun et al., 2014).

### *Telfairia occidentalis* (Cucurbitaceae)

This is an herbal plant cultivated mostly in the West African sub-region (Burkill, 1995). The leave extract of the plant is used locally in the treatment of malaria and anaemia (Gbile, 1986). Apart from its nutritional (Okoli and Mgbeogu, 1983), agricultural and industrial importance (Akoroda, 1990), the plant is also medicinally useful. It possesses anti-inflammatory (Oluwole et al., 2003), antibacterial (Odoemena and Essien, 1995), erythropoietic (Ajayi et al., 2000), anticholesterolemic (Eseyin et al., 2005a) and antidiabetic activities (Eseyin et al., 2005b, Ekpenyong et al., 2012). The ripe fruit

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contains up to 13% oil. The leaves and the young shoots of the plant are frequently eaten as a potherb (Ajao and Akindele, 2013). The seeds of the plant are also popular items of diet and are cooked whole and ground up into soups (Dina et al., 2006). The leaves also contain protein, vitamins, and flavours (Ekpenyong et al., 2012; Ajao and Akindele, 2013). In Nigeria, the herbal preparation of the plant has been employed in the treatment of sudden attack of convulsion, malaria and anaemia (Dina et al., 2006).

### ***Parquetina nigrescens* (Periplocaceae)**

This is a shrub found in Equatorial West Africa and has been in use in traditional medicine practice for centuries (Burkett, 1968). In Oyo State Nigeria, the leaves have been reputed for treatment of helminthiasis (intestinal worm) while the roots are reputed for use as an antirheumatic [oral communications]. Over the years, the leaf and root decoctions of *P. nigrescens* have been used for the treatment of gonorrhea and menstrual disorders (Schlage, 2002). The whole plant is used to stupefy fish in Ghana and Liberia, while the leaves and latex are used for the treatment of rickets, diarrhoea, skin lesions and tropical skin diseases (Schlage, 2002). The leaves of the plant have been used for the treatment of wounds, boils, and carbuncles in Africa (Agyare et al., 2009). *P. nigrescens* is also a constituent of a commercial herbal preparation (Jubi formular®) in Nigeria which is used in the treatment of sickle cell anaemia in human (Imaga et al., 2010). The Jubi formular was shown to restore decreased haematocrit and haemoglobin concentration in *Trypanosoma brucei* induced anaemia (Erah et al., 2003). Similarly, anti-sickling property of the root and leaves of *P. nigrescens* was confirmed by Kade et al. (2003) while the whole plant was investigated by Imaga et al. (2010). Agbor et al. (2001) also investigated and confirmed the antianaemic activity of aqueous extracts of *P. nigrescens* leaf on haemorrhagic anaemia induced in rats (Agbor and Odetola, 2001). Also, Akinyemi and Dada (2013, 2014) reported the anti-typhoid activity of ethanolic leaf extract of *P. nigrescens* in mice (Akinyemi and Dada, 2013; Akinyemi and Dada, 2014). The methanol leaf and other aerial parts extracts, and root extract of *P. nigrescens* have been shown to exhibit dose and time dependent toxicity in animals (Louis Adu-Amoaha et al., 2014; Owoyele et al., 2011).

Although, several investigations have been conducted on *C. sinensis*, *T. occidentalis* and *P. nigrescens* leaves as foods and herbal remedies, there is dearth of information on the safety evaluation of these plants as biological agents for radio-protection in experimental models. This study therefore, seeks to evaluate the acute and sub-acute toxicity potentials of aqueous leaves extracts of *C. sinensis*, *P. nigrescens* and *T. occidentalis* in mice.

## **MATERIALS AND METHODS**

### **Materials, apparatus and reagents**

These includes analytical balance (Golden-Mettler, U. S. A.), normal saline, formalin buffer, fixative (4%) paraformaldehyde, heamatoxylin/eosin, oral cannula, eppendorf, spatula, syringes (1, 2, 5, and 10 mL), laboratory wares and consumables.

### **Collection and identification of the plant material**

Fresh leaves of *P. nigrescens* and *T. occidentalis* were collected from the Plant Garden of African Centre for Herbal Research Institute, University of Ilorin while a refined product of *C. sinensis* was purchased from pharmaceutical premises in Ilorin. The plants were identified and authenticated by a taxonomist of the Department of Plant Biology, University of Ilorin, Nigeria. *P. nigrescens* was given Serial Number 876 and Ledger Number 67 while *T. occidentalis* was given Serial Number 959 and Ledger Number 150. Thereafter, collected samples were deposited in the herbarium of the institution for future reference.

### **Extraction of the plants materials**

Four hundred grams and 350 g of the powdered leaves of *T. occidentalis* and *P. nigrescens* respectively were each soaked in distilled water in a closable container. The finished product (fine granules) of *C. sinensis* was also weighed (500 g) and soaked in distilled water. These were shaken for about 5 min and left to extract by means of maceration (shaking the mixture intermittently) at 28°C for 72 h. The mixtures were filtered into a porcelain crucible using a fine mesh. The supernatant was concentrated below 40°C using rotary evaporator and then freeze-dried. The extract was stored at 4°C in freeze-dried form and used for the toxicity experiments later.

### **Preliminary phytochemical screening**

The phytochemical constituents of the aqueous extract were determined using standard procedures described by Sofowora (2008) and Trease and Evans (2009). The extracts were tested for the presence or absence of saponins, tannins, alkaloids, anthraquinones, cardiac glycosides, flavonoids and terpenoids.

### **Brine shrimp lethality (BSL) bioassay**

The eggs of Brine shrimp (*Artemia salina*) were obtained from Pharm., Kayode M. Salawu and hatched in natural seawater obtained from the Bar Beach, Ikoyi, Lagos and incubated for 48 h in 3.8 g/L seawater. The assay was the method described by McLaughlin (1991). Ten milligrams (10 mg) of three extracts were diluted to 1000 µg/mL by adding the sea water. Serial dilutions of the extracts were made in 96-well microplates in triplicates. Negative control wells contained sea water, while cyclophosphamide was used as the positive control. A 250 µL suspension of nauplii in the extract was added to each well. The plates were incubated at room temperature (25°C) for 24 h. The number of dead nauplii in each well was counted.

Data obtained was analyzed by computer program (Graphpad prism version 6.00). The concentration with 50% lethality (LC<sub>50</sub>) was calculated by nonlinear regression analysis.

## Ethical approval for animal studies

Ethical clearance and approval for the toxicity studies in mice was given by the University of Ilorin Ethical Review Committee, Ilorin, Nigeria in accordance with the Guide for Care and Use of Laboratory Animals, NIH, Department of Health Services Publication, USA, no. 83-23, revised 1985.

## Animals

Healthy Swiss male albino mice (17-27 g) were selected for both the acute and sub-acute toxicity studies and kept in plastic cages ( $34 \times 47 \times 18 \text{ cm}^3$ ) in the animal room of the Toxicology Unit, Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Ilorin, Ilorin, Nigeria. The animals were kept in an air conditioned environment with three mice in each cage and maintained at room temperature of  $25 \pm 2^\circ\text{C}$  with relative humidity ( $60\% \pm 10\%$ ) under 12 h night and light cycle. The animals used for the experiment were approved by Animal Ethics Committee of the University. They had free access to standard pellets as basal diet and water *ad libitum*. Animals were habituated to laboratory conditions for two weeks prior to experimental protocol to minimize if any of non-specific stress. All experimental procedures were conducted as stipulated by the Committee as well as internationally accepted guidelines for laboratory animal use and care.

## Acute toxicity procedure

Acute toxicity studies of aqueous extracts of *C. sinensis*, *P. nigrescens* and *T. occidentalis* were carried out in male mice using Lorke's method ( $n=3$ ). Twenty-seven mice were grouped into equal nine groups. Following an overnight fast, the mice were weighed and the doses were calculated in reference to their body weights. The first three groups received aqueous extract of *C. sinensis* (500, 1000 and 2000 mg/kg.), the second three groups received aqueous extracts of *P. nigrescens* (1000, 2000 and 4000 mg/kg) while the third three groups received the aqueous extract of *T. occidentalis* (1250, 2500 and 5000 mg/kg). All the mice were observed for general behavioral changes; symptoms of toxicity and mortality after treatment for the first four (critical) hours, then further observations were made every 8 h for 24 h. The absence of death of any animal in this phase was a pre-requisite to proceed to the second phase.

In the second phase, 9 mice were grouped into three of one mouse each. The first group received aqueous extract of *C. sinensis* (1000, 2000 and 4000 mg/Kg), the second group received aqueous extracts of *P. nigrescens* (3000, 6000 and 12000 mg/Kg) while the third group received the aqueous extract of *T. occidentalis* (2500, 5000 and 10000 mg/Kg). The animals were observed critically for about 30 min for signs of toxicity or mortality and further observations were made every 8 h for 24 h. Further critical observation of all the mice were made for a period of 14 days.

## Sub-acute toxicity studies

Sub-acute toxicity study (28-day repeated oral toxicity study) was carried out based on the calculated  $\text{LD}_{50}$ . Mice were divided into ten groups with 5 animals per group. Group I received distilled water orally at a dose of 10 ml/kg body weight and served as the control group whereas Groups II, III and IV received *C. sinensis* at dose rates of 700, 1400 and 2800 mg/kg respectively. Groups V, VI and VII received *P. nigrescens* at dose rate of 2000, 4000 and 8000

mg/kg respectively, while Groups VIII, IX and X received *T. occidentalis* at 1750, 3500 and 7000 mg/kg body weight respectively. All the groups of mice were observed twice daily for mortality and morbidity till the completion of the experiment. All the animals were observed for clinical signs and the time of onset and the duration of these symptoms were recorded. Body weights of the mice in all groups were recorded once before the start of dosing, once weekly during the treatment period and finally on the day of sacrifice. The amount of food intake was recorded every day and the data were expressed as 7 days cumulative value. At the end of the experiment (on the 29th day), following an overnight fast of 8 h (only water allowed) prior to necropsy and blood collection, all animals in various groups were anaesthetized under chloroform prior to euthanization and then decapitated by cervical dislocation. Blood samples were collected by cardiac puncture into ethylene diamine tetra acetic acid (EDTA-2K) and plain containers for haematological and biochemical investigations respectively. Blood in plain containers were allowed to clot and centrifuged and serum was collected for biochemical analyses.

## Hematological parameters

EDTA blood was used for the measurement of hemoglobin, red blood cell count, white blood cell count, platelet count using fully automated haematology analyzer (Sysmex KX 21N).

## Biochemical parameters

The serum was used to measure sodium, potassium [Ion Selective Electrodes], while urea, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), triglycerides, total cholesterol and total protein were determined using fully automated biochemical analyzer (Chemray 240, India).

## Histopathology

Following blood collection on day 29, all the animals were euthanized for gross pathological examinations of all major internal organs. Organs of interest [liver, kidney, brain, stomach, heart, testis, lung and spleen] were dissected, immediately cleaned of blood using physiological saline, weighed and preserved in 10% neutral buffered formalin. Tissues embedded in paraffin wax were sectioned 5  $\mu\text{m}$  thick, stained with haematoxylin and eosin, mounted on glass slides and examined under a standard light microscope for histopathological study.

## Statistical analysis

Results were expressed as mean  $\pm$  standard error of mean (SEM). Data obtained was analyzed using one way ANOVA followed by Tukey HSD. P values  $<0.05$  was considered as statistically significant.

## RESULTS

All the three aqueous extracts tested showed the presence of saponins, tannins, alkaloids, cardiac glycosides, flavonoids, and terpenoids. However, anthraquinones (both free and combined form) are present in *P. nigrescens* and *T. occidentalis* but absent in

**Table 1.** Preliminary phytochemical screening.

| Bioactive constituent | Chemical test     | <i>Telfairia occidentalis</i> | <i>Parquetina nigrescens</i> | <i>Camellia sinensis</i> |
|-----------------------|-------------------|-------------------------------|------------------------------|--------------------------|
| Alkaloid              | Drangendorff's    | +                             | +                            | ++                       |
|                       | Wagner's          | +                             | +                            | ++                       |
|                       | Meyer's           | +                             | +                            | +++                      |
| Flavonoid             | Shinoda's test    | ++                            | +++                          | +++                      |
|                       | Lead Ace Tate     | ++                            | +++                          | +++                      |
| Tannins               | FeCl <sub>3</sub> | +                             | +                            | +++                      |
| saponins              | Frothing          | ++                            | +                            | +++                      |
|                       | Emulsifying       | ++                            | +                            | +++                      |
|                       | Haemolysis        | +++                           | +                            | +++                      |
| Anthraquinone         | Free              | +                             | +                            | —                        |
|                       | Combined          | ++                            | +                            | —                        |
| Cardiac Glycoside     | Keller Killiani   | +++                           | +++                          | ++                       |
|                       | Kedde             | ++                            | ++                           | ++                       |
| Steroid Terpenoid     | Salkowski         | +++                           | +++                          | +++                      |

—, Absence of component; +, Trace presence of component; ++, moderate amount of component, +++, Copious amount of component.

*C. sinensis* as shown in Table 1.

### Acute toxicity studies

Oral administration of extracts of *C. sinensis* (500, 1000 and 2000), *P. nigrescens* (1000, 2000 and 4000 mg/kg), *T. occidentalis* (1250, 2500 and 5000 mg/kg) produced no deaths or clinical signs of toxicity in mice. However, there were mortality and clinical signs of toxicity at 4000, 12,000 and 10000 mg/kg body weight for *C. sinensis*, *P. nigrescens* and *T. occidentalis* respectively. The LD<sub>50</sub> for *C. sinensis*, *P. nigrescens* and *T. occidentalis* extracts were 2828.43, 8485.28 and 7071.07, mg/kg respectively. Thus, convenient doses were chosen for sub-acute toxicity studies to preclude the lethal range.

### Sub-acute toxicity studies

There were no treatment related toxicity signs or mortality observed among mice treated at 700, 1400 and 2800 mg/kg for *C. sinensis*; 2000, 4000 and 8000 mg/kg for *P. nigrescens* and 1750, 3500 and 7000 mg/kg for *T. occidentalis* during the 4 weeks of treatment.

### DISCUSSION

Medicinal plants were and are still one of the major sources of modern medicine. The general belief that

herbal products prepared from medicinal plants are safe since they are from natural sources are not always correct. Interest in medicinal plant's pharmacognosy has increased due to trend of phytotherapy as alternative to orthodox medicine. With the increased interest in the pharmacological activities of the medicinal plants, there is a reason for thorough scientific investigations of these medicinal plants for efficacy and potential toxicity. Proper scientific evidence is necessary to establish toxicological profile of commonly used medicinal plants as safe, non-toxic and pharmacologically active. Acute and sub-acute safety evaluation of plants extracts are required as an effective parameter for calculating the therapeutic index of drugs and chemicals, to identify the further range of doses in animal studies and to explain the probable clinical signs evoked by the test compounds under investigation (Akhila et al., 2007). Results obtained from toxicity studies on animals will be critical for positive judgement on the safety of medicinal plants if they are found to have adequate potential for development into pharmacological compounds (Chang, 1987). Against this background, the present study evaluated the acute and sub-acute toxicity of aqueous leaves extracts of *C. sinensis*, *P. nigrescens* and *T. occidentalis* in mice using standard toxicological procedures. Several studies however, conducted on these plants have established numerous medicinal properties and health benefits. Aqueous leaves extracts of the three plants have well-documented evidence-based findings of promotive, preventive, corrective and curative potentials. Towards

**Table 2.** Brine shrimp lethality of *C. sinensis*, *P. nigrescens* and *T. occidentalis* extracts.

| Concentration (µg/mL)          | Percentage lethality |                      |                        |                  |
|--------------------------------|----------------------|----------------------|------------------------|------------------|
|                                | <i>C. sinensis</i>   | <i>P. nigrescens</i> | <i>T. occidentalis</i> | Cyclophosphamide |
| 1000                           | 100                  | 100                  | 100                    | 100              |
| 500                            | 100                  | 100                  | 100                    | 80               |
| 100                            | 10                   | 80                   | 90                     | 50               |
| 10                             | 0                    | 20                   | 50                     | 10               |
| 1                              | 0                    | 0                    | 0                      | 0                |
| <b>LC<sub>50</sub> (µg/mL)</b> | <b>418.6</b>         | <b>32.34</b>         | <b>8.32</b>            | <b>94.76</b>     |

achieving their safety profiles, gross behavioural assessment; body/organ weight; food intake; haematological, and biochemical parameters; and histomorphology of major vital organs were evaluated.

The preliminary phytochemical assay was conducted to determine the presence of plant metabolites in all the three samples under study. The three aqueous extracts tested depicted that none of them contains toxic plant metabolites rather, tannins, alkaloids, cardiac glycosides, flavonoids, saponins, and terpenoids were present (Tables 1, 3, 4 and 5). Since all the secondary metabolites present are non-toxic type, perhaps this justified the absence of any significant morbidity and lethality in all the groups in both acute and sub-chronic toxicity studies. Otherwise, significant morbidity and perhaps lethality might have been witnessed if compounds like anthocyanins were to be present, even though the mild toxic signs observed in groups administered high doses of *T. occidentalis* (10000 mg/kg) and *P. nigrescens* (12,000 mg/kg) may likely be attributed to the copious presence of anthraquinones (both free and combined form) in *P. nigrescens* and *T. occidentalis*, respectively.

Brine shrimp lethality (BSL) model has proved helpful as a preliminary screening model in the drug design and synthesis of cytotoxic compounds (Nazir et al., 2013). The lethality of the extracts on the brine shrimps was classified according to the method of Padmaja et al. (2002). Where  $LC_{50} \geq 1000$  µg/mL was considered to be non-toxic,  $LC_{50} = 500$  to  $1000$  µg/mL as weakly toxic,  $LC_{50} = 100$  to  $500$  µg/mL as moderately toxic and  $LC_{50} \leq 100$  µg/mL as strongly toxic. Only extract of *C. sinensis* ( $LC_{50}=418.6$  µg/mL) was slightly more toxic on the brine shrimps, while *P. nigrescens* ( $LC_{50}=32.34$  µg/mL) and *T. occidentalis* ( $LC_{50}=8.32$  µg/mL) were observed to be strongly toxic on the brine shrimps (Table 2).

The  $LD_{50}$  of the extract were low for *C. sinensis*, but high for *P. nigrescens* and *T. occidentalis* (2828.43, 8485.28 and 7071.07 mg/kg respectively). Following the establishment of  $LD_{50}$  for each of the plant, the sub-acute dose ranges of low, moderate and high doses were assigned to each of the extract (Tables 3 to 5). The results showed no significant treatment-related signs or deaths in the graduated doses in all the plant: *C. sinensis* (700, 1400 and 2800 mg/kg), *P. nigrescens* 2000, 4000

and 8000 mg/kg) and *T. occidentalis* (1750, 3500 and 7000 mg/kg) throughout the 28-day of the administration. The *T. occidentalis* group showed no signs of acute toxicity, rather they exhibited calm behavior and ptosis. This finding is similar to the finding of Ekpenyong (2012). Contrarily, the animals administered doses of *C. sinensis* extract showed signs of hyperactivity, irritable behavior, and agitation within 2 h post-administration, however, with no clinical signs of toxicity. Behavioral manifestations observed for 2 h post-oral treatment of all dose levels of aqueous leaf extract of *P. nigrescens* included reduced locomotion, calmness, writhing effects, passivity and hypoactivity.

Generally speaking, there were no statistically significant differences recorded in body weight, food intake and relative organ weights in all the study groups. However, food intake was observed to decrease proportionately with decrease body weight when compared with the control group, albeit not statistically significant (Figure 1 to 3). These findings of poor food intake and reduced body weight may probably be attributed to the low intestinal uptake (bioavailability) of the extracts and green tea (*C. sinensis*) in particular because absorption of *C. sinensis* is improved on an empty stomach as previously reported by Chen et al. (1997) and Naumovski et al. (2015). It could also support the notion that green tea can be more effective in reducing weight in some populations (Brown et al., 2011).

Further, treatment with aqueous leaf extracts for 28 days significantly increase haemoglobin, red blood cell counts and packed cells volume as observed in groups administered *C. sinensis* and *T. occidentalis* but, contrary to the group given *P. nigrescens* when compared to the control group (Tables 6 to 8). Similarly, there were significant increments in leucopoiesis in groups given moderate and high doses of *C. sinensis* and in low and moderate doses of *T. occidentalis* and *P. nigrescens* (Tables 6 to 8). Megakaryopoiesis was also significantly increased in low and moderate doses of *C. sinensis* and *T. occidentalis*, in agreement with previous reports (Eseyin et al., 2005a; Park et al., 2003; Sakata et al., 2004; Dina et al., 2006).

Transaminases (AST and ALT) are well known good indicators of liver function and are used as biomarkers to show probable toxicities of drugs and xenobiotics.

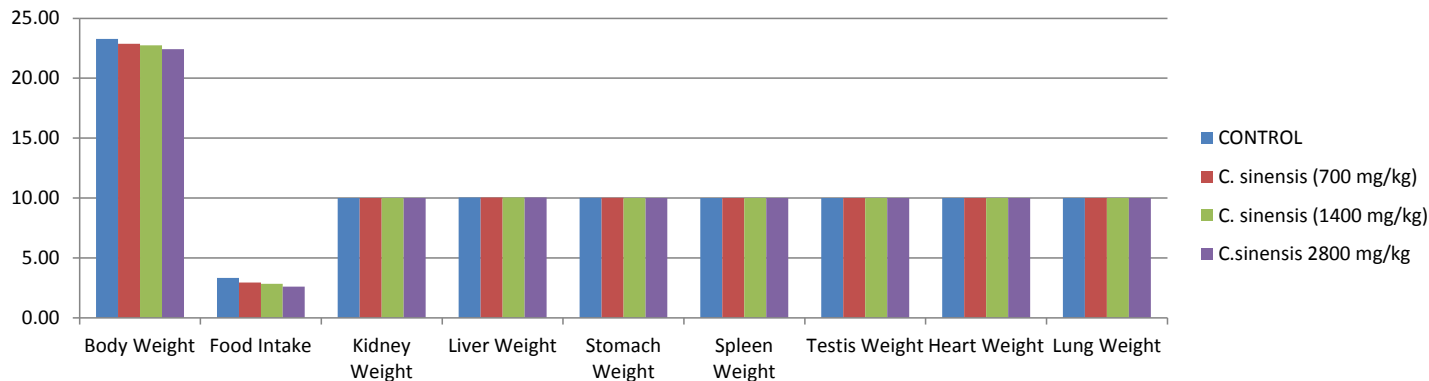


**Figure 1.** Mean of the effect of aqueous leaves extract of *Camellia sinensis* on food intake (g), body weight gain (kg) and relative organ weight (g) in mice.

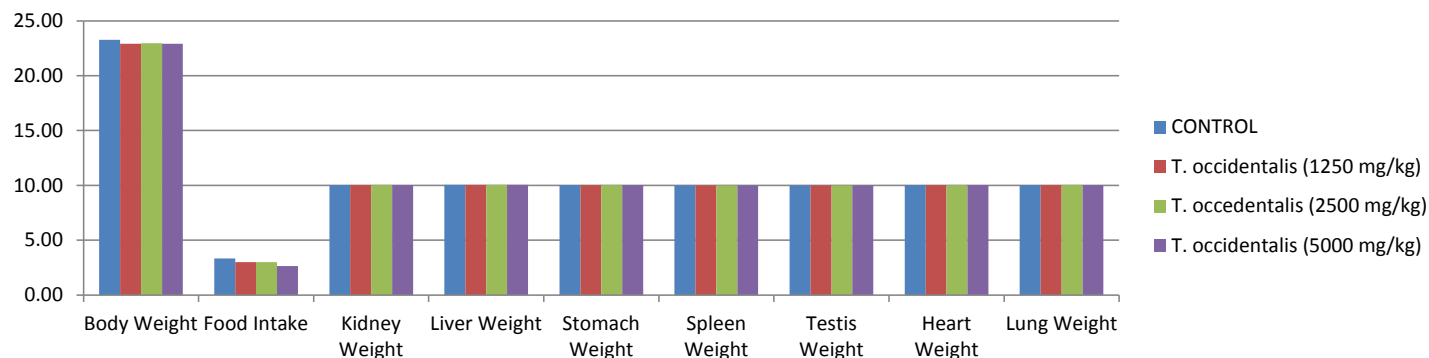


**Figure 2.** Mean of the effect of aqueous leaves extract of *Telfairia occidentalis* on food intake (g), body weight gain (kg) and relative organ weight (g) in mice.

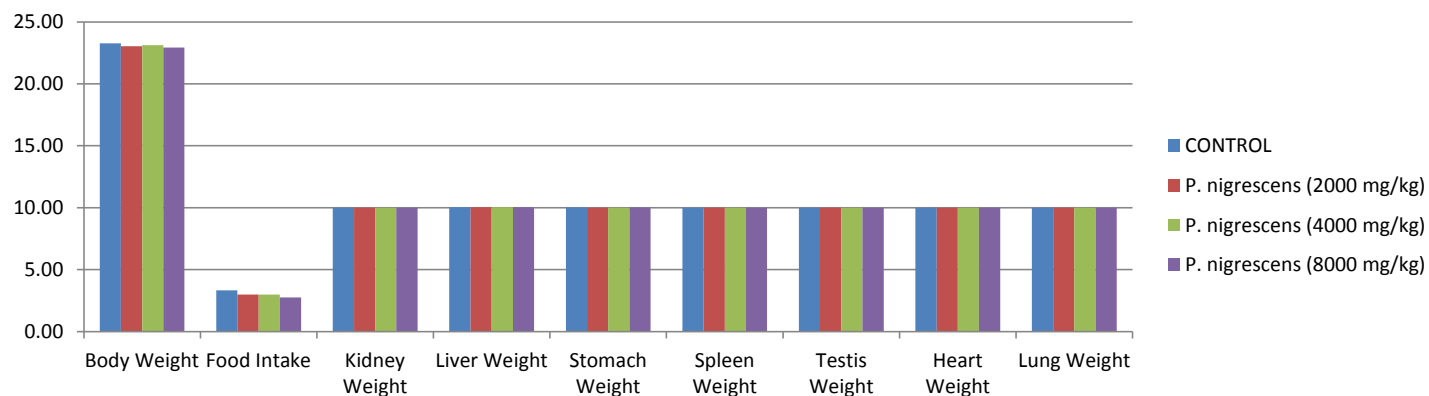


**Figure 3.** Mean of the effect of aqueous leaves extract of *Parquetina nigrescens* on food intake (g), body weight gain (kg) and relative organ weight (g) in mice.

Usually, destruction of the liver parenchymal cells results in an increase in both AST/ALT enzymes in the blood (Odoemena et al., 1995; Ajayi et al., 2000). In this study however, no significant changes in the ALT/AST/ ALP and total protein levels were reported in various doses of

the extracts. Nonetheless, significant increase in AST/ALT was observed in the group administered high dose of *P. nigrescens* only. Although, cholesterol has not significantly changed, triglycerides were significantly lowered with low doses of *C. sinensis* and high dose of *P.*

**Table 3.** Acute oral toxicity of leaves aqueous extracts of *C. sinensis*.

| <i>C. sinensis</i>     | Phase 1        |           | Phase 2        |           |
|------------------------|----------------|-----------|----------------|-----------|
|                        | Dose (mg/kgbw) | Mortality | Dose (mg/kgbw) | Mortality |
| Low                    | 500            | 0/3       | 1000           | 0/3       |
| Moderate               | 1000           | 0/3       | 2000           | 0/3       |
| High                   | 2000           | 0/3       | 4000           | 2/3       |
| <b>LD<sub>50</sub></b> | <b>2828</b>    |           |                |           |

**Table 4.** Acute oral toxicity of leaves aqueous extracts of *P. nigrescens*.

| <i>P. nigrescens</i>   | Phase 1        |           | Phase 2        |           |
|------------------------|----------------|-----------|----------------|-----------|
|                        | Dose (mg/kgbw) | Mortality | Dose (mg/kgbw) | Mortality |
| Low                    | 1000           | 0/3       | 3000           | 0/3       |
| Moderate               | 2000           | 0/3       | 6000           | 0/3       |
| High                   | 4000           | 0/3       | 12000          | 2/3       |
| <b>LD<sub>50</sub></b> | <b>8485</b>    |           |                |           |

**Table 5.** Acute oral toxicity of leaves aqueous extracts of *T. occidentalis*.

| <i>T. occidentalis</i> | Phase 1        |           | Phase 2        |           |
|------------------------|----------------|-----------|----------------|-----------|
|                        | Dose (mg/kgbw) | Mortality | Dose (mg/kgbw) | Mortality |
| Low                    | 1250           | 0/3       | 2500           | 0/3       |
| Moderate               | 2500           | 0/3       | 5000           | 0/3       |
| High                   | 5000           | 0/3       | 10000          | 1/3       |
| <b>LD<sub>50</sub></b> | <b>7071</b>    |           |                |           |

*nigrescens*. Electrolytes (Na<sup>+</sup> and K<sup>+</sup>) were not significantly affected in all the groups except with the group administered high doses of *T. occidentalis* and *P. nigrescens*. Nevertheless, urea and creatinine were not significantly affected in all the groups (Tables 9 to 11).

Moreso, histopathological studies provide supportive evidence for biochemical and haematological observations. Overall histopathological examinations and analyses of the major vital organs which include; liver, kidney, heart, lungs, stomach, spleen and testes showed a well preserved cytoarchitecture. Specific features were assessed which include but not limited to scarring (cirrhosis), necrosis, inflammation, sinusoidal dilatation, haemorrhage, hypercellularity, fibrosis, lymphocytic infiltration. Various doses of all the groups did not only show no toxicity but equally showed well preserved tissue morphology. Notwithstanding, *P. nigrescens* at high doses showed mild kidney interstitial fibrosis, mild glomerular hypercellularity (mesangial proliferation), mild inflammation of the intestines, microhemorrhages and mild liver changes (mild inflammation, mild central vein fibrosis, mild central vein inflammation).

Similarly, testes were well preserved with complete

sequence of seminiferous tubules, normal germ cells, normal leydig cells as well intact basement membrane. These mild tissues derangement corroborated the biochemical findings of slightly increased values of AST and ALT observed in this group. Previous study of ethanolic extracts of *P. nigrescens* in rats reported renal haemorrhage and inflammation and hepatic inflammation in low doses, while high dose administration showed restoration of glomerular tufts and improved hepatic vasculature with reduced inflammatory infiltrates (Feng et al., 2001). This however, contradicts the study findings since groups administered various doses of *P. nigrescens* did not reveal any end organ toxicity as assessed by histological and biochemical analyses. Also, Ajao and Akindele (2013) reported no signs of delayed toxicity or death with very high oral doses of *T. occidentalis* which is similar to reports made by Chow et al. (2003), Yu et al. (2011) and Ekpenyong et al. (2012). However, administration of high doses either Teavigo or Polyphenon E (two brands of green tea catechins) to beagle dogs resulted in dose-dependent toxicity with vomiting and diarrhea, resulting in death. It is noteworthy that, eagle dogs have better absorption rates of green

**Table 6.** Mean±SEM of the effect of varying doses of aqueous leaf extract of *C. sinensis* on haematologic parameters in mice.

| Group      | Hb (g/dl)   | PCV (%)     | RBC× 10 <sup>12</sup> | MCV (fl)   | MCH (pg)   | MCHC (g/dl) | WBC × 10 <sup>9</sup> | PLT × 10 <sup>12</sup> | NEUT (%)   | LYMP (%)   | EOSIN (%) | MONO (%)  | BASO (%)  |
|------------|-------------|-------------|-----------------------|------------|------------|-------------|-----------------------|------------------------|------------|------------|-----------|-----------|-----------|
| Control    | 12.00±0.22  | 36.00±0.71  | 4.42±0.12             | 84.20±2.76 | 28.80±0.86 | 34.20±0.49  | 3.56±0.14             | 347.80±19.58           | 11.40±1.72 | 86.40±1.86 | 2.20±0.66 | 0.00±0.00 | 0.00±0.00 |
| 700 mg/kg  | 14.88±0.55* | 43.20±1.62* | 5.25±0.17*            | 87.60±2.71 | 27.40±0.98 | 32.40±1.08  | 4.28±0.15             | 421.20±12.03*          | 16.20±2.62 | 81.20±3.15 | 2.20±0.49 | 0.60±0.40 | 0.00±0.00 |
| 1400 mg/kg | 15.90±0.33* | 46.20±1.02* | 5.55±0.12*            | 92.40±1.86 | 30.80±2.20 | 31.60±1.03  | 5.96±0.25*            | 457.00±17.78*          | 9.20±0.49  | 88.80±0.49 | 2.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| 2800 mg/kg | 13.70±0.27* | 41.80±0.80* | 5.06±0.08*            | 83.60±2.94 | 28.00±1.14 | 33.40±1.12  | 5.38±0.49*            | 406.00±12.84           | 17.80±1.50 | 78.80±1.46 | 2.60±0.40 | 0.60±0.00 | 0.20±0.20 |

The Hb, PCV and RBC were significantly increased in all the doses with moderate dose showing the highest value. WBC was significantly increased at moderate and high doses, PLT significantly increased at low and moderate doses. Values are expressed as Mean±SEM (n=5). \* indicates significant difference when compared to Control, at p< 0.05.

**Table 7.** Mean±SEM of the effect of varying doses of aqueous leaf extract of *T. occidentalis* on haematologic parameters in mice.

| Group      | Hb (g/dl)   | PCV (%)     | RBC× 10 <sup>12</sup> | MCV (fl)   | MCH (pg)   | MCHC (g/dl) | WBC × 10 <sup>9</sup> | PLT × 10 <sup>12</sup> | NEUT %     | LYMP %     | EOSIN %   | MONO %    | BASO %    |
|------------|-------------|-------------|-----------------------|------------|------------|-------------|-----------------------|------------------------|------------|------------|-----------|-----------|-----------|
| Control    | 12.00±0.22  | 36.00±0.71  | 4.42±0.12             | 84.20±2.76 | 28.80±0.86 | 34.20±0.49  | 3.56±0.14             | 347.80±19.58           | 11.40±1.72 | 86.40±1.86 | 2.20±0.66 | 0.00±0.00 | 0.00±0.00 |
| 1250 mg/kg | 14.41±0.52* | 42.60±1.08* | 5.22±0.11*            | 83.80±1.72 | 29.20±0.80 | 34.00±0.63  | 4.98±0.28*            | 442.80±8.14*           | 12.60±1.60 | 86.00±1.76 | 1.40±0.25 | 0.00±0.00 | 0.00±0.00 |
| 2500 mg/kg | 14.67±0.22* | 44.00±0.71* | 5.49±0.12*            | 91.60±1.75 | 29.20±0.58 | 31.40±1.12  | 6.50±0.16*            | 485.20±8.49*           | 11.60±1.03 | 86.80±1.24 | 1.60±0.40 | 0.00±0.00 | 0.00±0.00 |
| 5000 mg/kg | 14.20±0.42* | 41.80±0.80* | 5.07±0.10*            | 82.80±2.40 | 29.20±0.58 | 34.40±0.68  | 4.50±0.22             | 431.60±21.01*          | 13.60±2.46 | 86.40±2.64 | 2.00±0.32 | 0.00±0.00 | 0.00±0.00 |

The Hb, PCV, RBC were significantly increased in all the doses. WBC and PLT were significantly increased consistently at low and moderate doses with moderate doses showing the highest value. Values are expressed as Mean±SEM (n=5). \* indicates significant difference when compared to control, at (p< 0.05).

**Table 8.** Mean±SEM of the effect of varying doses of aqueous leaf extract of *C. sinensis* on biochemical parameters in mice.

| Group      | Na (mmol/L) | K (mmol/L) | ALP (mmol/L) | ALT (mmol/L) | AST (mmol/L) | UREA (mmol/L) | CREAT. (mmol/L) | CHOL. (mmol/L) | TRIGLY (mmol/L) | T.PROTEIN (mmol/L) |
|------------|-------------|------------|--------------|--------------|--------------|---------------|-----------------|----------------|-----------------|--------------------|
| Control    | 135.80±3.90 | 7.20±0.13  | 51.80±2.48   | 145.20±3.28  | 228.80±7.53  | 2.40±0.16     | 34.00±1.30      | 1.58±0.11      | 0.90±0.05       | 50.60±1.91         |
| 700 mg/kg  | 132.80±1.36 | 6.82±0.28  | 43.00±1.52   | 133.00±1.62  | 206.20±3.80  | 2.50±0.15     | 33.60±1.57      | 1.58±0.07      | 0.58±0.07       | 43.60±1.63         |
| 1400 mg/kg | 143.40±1.54 | 7.02±0.22  | 46.20±1.28   | 143.20±2.22  | 208.20±2.76  | 2.26±0.12     | 33.40±1.08      | 1.62±0.09      | 0.80±0.03       | 49.20±1.39         |
| 2800 mg/kg | 147.00±3.32 | 6.90±0.07  | 46.20±1.36   | 133.20±1.93  | 215.40±13.69 | 2.28±0.10     | 33.20±1.39      | 1.58±0.09      | 0.70±0.05       | 44.40±1.33         |

The parameters were not statistically significant except significant decrease in Triglyceride at low dose. Values are expressed as Mean±SEM (n=5). \* indicates significant difference when compared to control, at p< 0.05.

tea catechins (Lambert et al., 2007). Vomiting may be associated with gastric damage with very

high oral doses to rats lead to 90% lethality associated with haemorrhagic lesions in the

stomach and intestines (Isbrucker et al., 2006). Interestingly, animals with lower absorption rates

**Table 9.** Mean±SEM of the effect of varying doses of aqueous leaf extract of *T. occidentalis* on biochemical parameters in mice.

| Group      | Na (mmol/L)  | K (mmol/L) | ALP (mmol/L) | ALT (mmol/L) | AST (mmol/L) | Urea (mmol/l) | Creat. (mmol/l) | Chol. (mmol/l) | Trigly. (mmol/L) | Total protein (mmol/L) |
|------------|--------------|------------|--------------|--------------|--------------|---------------|-----------------|----------------|------------------|------------------------|
| Control    | 135.80±3.90  | 7.20±0.13  | 51.80±2.48   | 145.20±3.28  | 228.80±7.53  | 2.40±0.16     | 34.00±1.30      | 1.58±0.11      | 0.90±0.05        | 50.60±1.91             |
| 1250 mg/kg | 144.20±2.69  | 6.44±0.15  | 42.40±2.11   | 132.20±3.31  | 204.40±8.93  | 2.42±0.17     | 36.00±1.38      | 1.70±0.05      | 0.76±0.08        | 44.80±1.50             |
| 2500 mg/kg | 141.00±1.10  | 6.26±0.11* | 42.40±3.78   | 139.80±5.95  | 212.20±4.59  | 2.48±0.18     | 33.00±1.41      | 1.68±0.05      | 0.80±0.03        | 50.20±3.01             |
| 5000 mg/kg | 151.40±5.67* | 6.62±0.27  | 49.80±2.35   | 137.00±3.33  | 206.20±4.96  | 2.64±0.14     | 35.60±0.75      | 1.66±0.08      | 0.82±0.02        | 43.80±1.16             |

The parameters were not statistically significant except Na<sup>+</sup> that was significantly high at high dose and K<sup>+</sup> that was significantly low at moderate dose. Values are expressed as Mean±SEM (n=5). \* indicates significant difference when compared to control, at p< 0.05.

**Table 10.** Mean±SEM of the effect of varying doses of aqueous leaf extract of *P. nigrescens* on biochemical parameters in mice.

| Group      | Na (mmol/L) | K (mmol/L) | ALP (mmol/L) | ALT (mmol/L) | AST (mmol/L)  | UREA (mmol/L) | CREAT. (mmol/L) | CHOL. (mmol/L) | TRIGLY. (mmol/L) | Total protein (mmol/L) |
|------------|-------------|------------|--------------|--------------|---------------|---------------|-----------------|----------------|------------------|------------------------|
| Control    | 135.80±3.90 | 7.20±0.13  | 51.80±2.48   | 145.20±3.28  | 228.80±7.53   | 2.40±0.16     | 34.00±1.30      | 1.58±0.11      | 0.90±0.05        | 50.60±1.91             |
| 2000 mg/kg | 131.80±3.88 | 6.42±0.13  | 45.40±1.50   | 142.20±4.41  | 193.80±2.06   | 2.46±0.17     | 36.80±1.16      | 1.64±0.08      | 0.80±0.03        | 43.20±1.59             |
| 4000 mg/kg | 142.20±0.58 | 6.34±0.16* | 37.40±1.94*  | 145.40±1.69  | 189.80±4.90   | 2.44±0.18     | 34.20±1.83      | 1.56±0.10      | 0.82±0.04        | 50.40±3.50             |
| 8000 mg/kg | 124.20±2.75 | 6.16±0.07* | 47.40±1.91   | 126.80±2.01* | 156.60±17.53* | 1.76±0.19     | 34.80±1.32      | 1.42±0.08      | 0.58±0.07*       | 46.80±0.86             |

The parameters were not statistically significant except significant reduction in K and ALP at moderate dose and K, ALT, AST and triglyceride that was significantly low at high doses. Values are expressed as Mean±SEM (n=5). \* indicates significant difference when compared to control, at p< 0.05.

**Table 11.** Mean±SEM of the effect of aqueous leaves extract of *C. sinensis* on food intake (g), body weight gain (kg) and relative organ weight (g) in mice.

| Group      | Food intake | Body weight | Kidney weight | Liver weight | Stomach weight | Spleen weight | Testis weight | Heart weight | Lung weight |
|------------|-------------|-------------|---------------|--------------|----------------|---------------|---------------|--------------|-------------|
| Control    | 3.33±0.00   | 23.27±0.56  | 0.01±0.00     | 0.04±0.00    | 0.03±0.00      | 0.01±0.00     | 0.00±0.00     | 0.01±0.00    | 0.01±0.00   |
| 700 mg/kg  | 2.95±0.05*  | 22.88±0.46  | 0.01±0.00     | 0.04±0.00    | 0.02±0.00      | 0.01±0.00     | 0.00±0.00     | 0.01±0.00    | 0.01±0.00   |
| 1400 mg/kg | 2.83±0.06*  | 22.74±0.24  | 0.01±0.00     | 0.04±0.00    | 0.01±0.00      | 0.01±0.00     | 0.00±0.00     | 0.01±0.00    | 0.01±0.00   |
| 2800 mg/kg | 2.61±0.07*  | 22.43±0.32  | 0.01±0.00     | 0.04±0.00    | 0.01±0.00      | 0.01±0.00     | 0.00±0.00     | 0.01±0.00    | 0.01±0.00   |

The extracts at different dose against all parameters did not show any significant difference when compared with control. However, Food intake at various doses was significantly decreased. Values are expressed as Mean±SEM (n=5). \*Indicates significant difference when compared to control at p< 0.05.

of epigallocatechin gallate (EGCG) suffered greater intestinal but less systemic damage (liver and kidneys) (Galati et al., 2006; Lambert et al.,

2007). More so, consumption (at moderate doses) of supplemental forms of Green Tea Catechins (*C. sinensis*) have been repeatedly found to be

safe (Chow et al., 2003; Ullmann et al., 2003; Ullmann et al., 2004).

In conclusion, the aqueous leaves extracts of *C.*

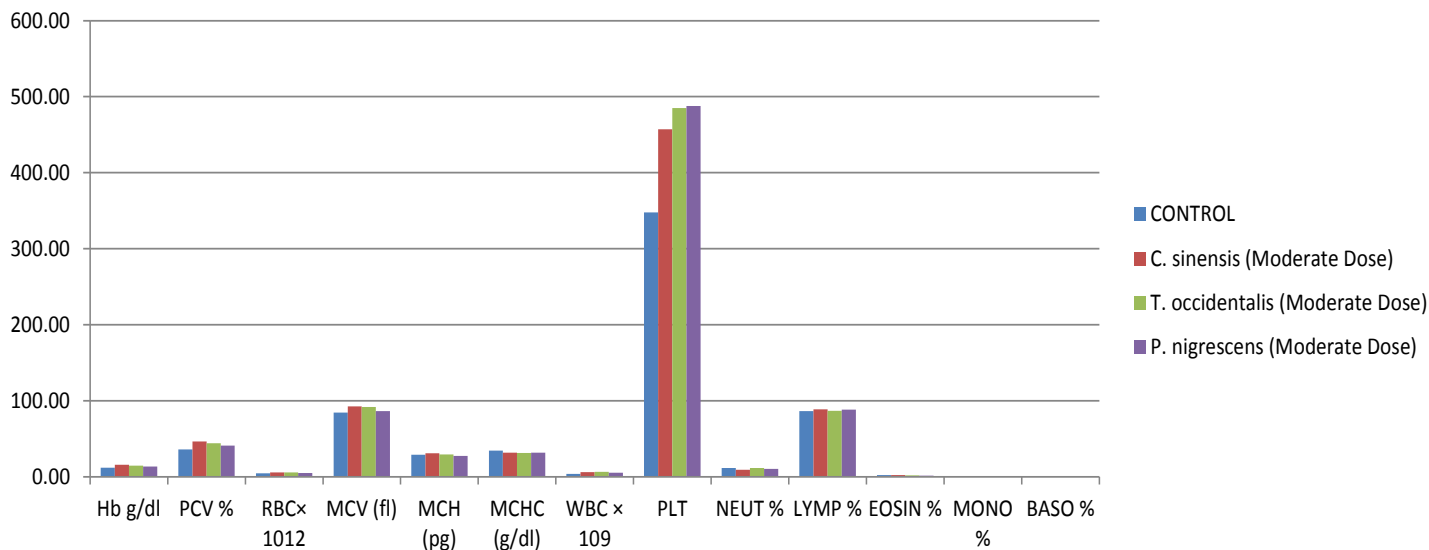


**Figure 4.** Comparative effects of *C. sinensis*, *T. occidentalis* and *P. nigrescens* moderate doses on haematologic parameters in mice.

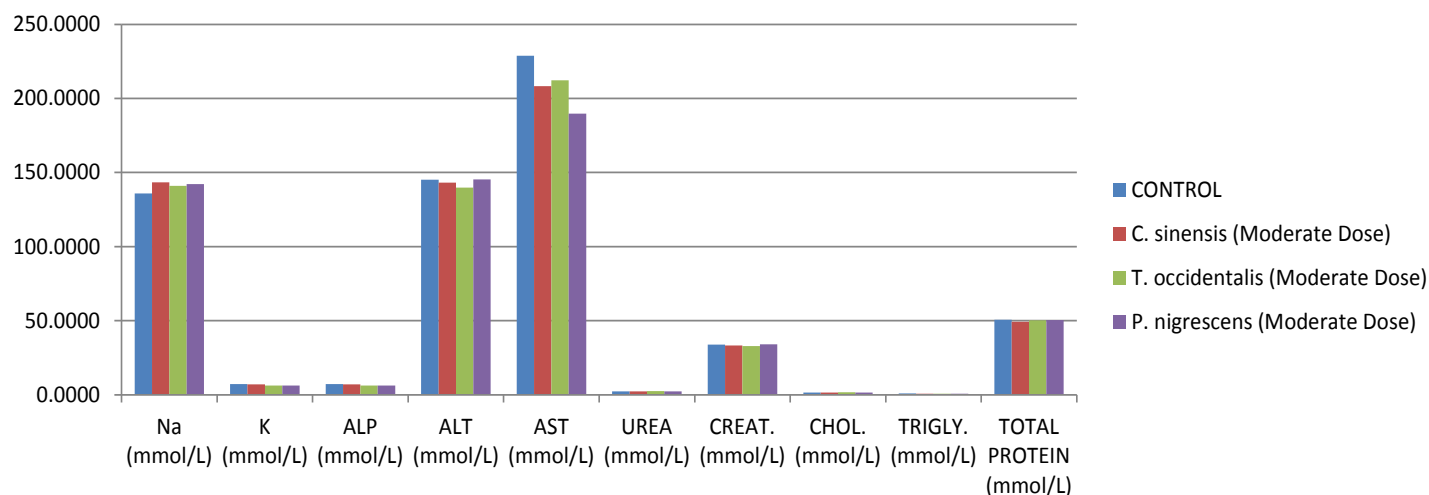


**Figure 5.** Comparative effects of *C. sinensis*, *T. occidentalis* and *P. nigrescens* moderate doses on biochemical parameters in mice.

*sinensis*, *T. occidentalis* and *P. nigrescens* are moderately safe as medicinal plants. This study established that all the three aqueous leaves extracts of these plants showed haemopoietic enhancement potential which is exhibited in a descending order: *T. occidentalis* > *C. sinensis* > *P. nigrescens* (Figures 4 and 5). Further studies to evaluate their potential roles in radiation-counter-effect, and determining their pharmacokinetic profiles in both animal and human subjects are thus, recommended.

#### CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

#### ACKNOWLEDGEMENT

The authors acknowledged the technical staff of Department of Pharmacognosy and Drug Development, University of Ilorin, Ilorin; and Department of Pharmacognosy, Obafemi Awolowo University, Ile-Ife, Nigeria; and the technologists at Central Science Laboratories, Obafemi Awolowo University, Ile-Ife, Nigeria for their roles in the collection, and extraction of plant materials. They also acknowledged the technical staff of the Department of Pharmacology and Toxicology, University of Ilorin, Ilorin for the animal handling and care; and Department of Medical Laboratory Services (Haematology, Chemical Pathology, and Histopathology Units), University of Ilorin Teaching Hospital, Ilorin for

samples analysis.

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