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Toxicities of *Parkia biglobosa* Extract and Dimethoate + Cypermethrin Insecticide on Kidney and Liver of Wistar Rats Fed Treated Okra Fruits

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Abstract

This study evaluated the sub-acute toxicities of *Parkia biglobosa* aqueous pod husk extract (PAPHE) and Dimethoate + Cypermethrin (D+C) insecticides treated okra fruits (milled) on the kidney and liver of wistar rats. Thirty-two male wistar rats randomly divided into eight experimental groups of four rats each were used for this study. The treatments were animals fed with: Standard Ratio Feed (SRF) + untreated okra (T1), SRF+ 2.5ml D+C okra treated (T2), SRF+5.0 ml D+C okra treated (T3), SRF + 20% PAPHE okra treated (T4), SRF+ 15% PAPHE (T5), SRF+10% PAPHE (T6), SRF+ 5% PAPHE (T7) and SRF only (Control 0%) (T8). Drinking water was given *ad libitum* for 21 days. Animal groups were sacrificed at the end of the experiment and vital organs were removed. Hematology and serum biochemical assays and histopathological identifications were done using standard procedures. Data were analyzed using ANOVA ($p<0.05$), while histopathology was examined. Results revealed no significant differences ($p>0.05$) amongst the treatments with the exception of treatment T5. The STR+15% PAPHE okra treated (T5) revealed highest values of platelets ($219x10^3/\mu L$), Mean Corpuscular Hemoglobin (21.61 g/dL), Globulin (4.57 g/dL), AST (45.67 U/L) and Creatinine (1.13 mg/dL). Treatment T5 was more toxic than the other treatments. Photomicrographs of sections of liver and kidney organs of the wistar rats showed lesions (necrosis) in all the treatments except the control groups fed on SRF and SRF + untreated okra and SRF+ okra treated with 5% PAPHE. As such, the aqueous pod husk extract of *P. biglobosa* (PAPHE) appeared to be safe for consumption at 5% concentration in agricultural sustainability for food quality and safety.

Keywords: *Parkia biglobosa* pod husk extract, Dimethoate+Cypermethrin, Okra, Wistar rats

1. Introduction

Okra (*Abelmoschus esculentus* (L.) Moench) is a fruit vegetable that is widely cultivated and consumed in Nigeria; it is cultivated for its fibrous fruits (or pods) and its leaves. It is a good source of carbohydrate, protein, fats, vitamins and minerals (Akintoye *et al.*, 2011; Ojo *et al*., 2014). Okra fruit can be cooked in a variety of ways; its leaves may also be cooked, eaten raw in salads or used as cattle feed (Fagwalawa and Yahaya, 2016). Mature fruits and stems of okra plant containing crude fiber are used in the paper industry (Moekchantuk and Kumar, 2004). Extracts from the seeds of the okra are viewed as an alternative source for edible oil. Okra is said to be very useful against genito-urinary disorders, spermatorrhoea and chronic dysentery (Robert *et al*., 2011). Its mucilage is also suitable for medicinal (curing ulcers and relief from hemorrhoids) and industrial applications (Akinyele and Temikotan, 2007).

Okra is prone to damage by various insects such as: *Podagrica uniforma* and *P. sjostedti*, others include *Anomis flava*, *Earias biplaga*, *Aphis gossypii*, *Aphis craccivora* (Kumar, 2010). Incidence of attacks on okra leaves starts as early as the germination stage by *Podagrica* species that create circular and irregular holes on okra leaves and punctures on pods. *Sylepta derogate* rolls okra leaves prior to pupation causing a reduction in the photosynthetic sites of the leaves (Ogbalu *et al*., 2015).

The exposure of synthetic pesticides (insecticides, fungicides, herbicides) to fruits and vegetable production has been causing adverse health implication and environmental pollution. Pesticides have deleterious effects on non-target species; they affect animal and plant biodiversity, food webs and the ecosystem (Mahmood *et al*., 2016). This problem led to a more sustainable approach to pest control and natural crop protection (botanicals) (Fayinminnu, 2010) with pesticidal potency. Pesticidal effects of many plants have been discovered and researched, such as Neem (*Azadirachta indica)*, *Derris elliptica*, Chilli pepper (*Capsicum annum*), Giant milkweed (*Calotropis procera*), Mint weed (*Hyptis suaveolens*), Tobacco (*Nicotiana tabacum*), Cassava (*Manihot esculenta*), Eggplant (*Solanum melongena*), African locust bean tree (*Parkia biglobosa*) (Salako *et al*., 2015), Purple weed (*Alternanthera brasiliana*) (Fayinminnu and Shiro, 2014) Drumestick (*Moringa oleifera*) (Fayinminnu *et al*., 2015). Phytochemicals inherent in *P. biglobosa* have increased their use as a

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biopesticide to suppress different insect pests of okra (Fayinminnu *et al*., 2017).

Biopesticides result in lower pesticide residues in food and environment, thereby preventing pollution problems and health hazards associated with synthetic pesticides; but toxic effects of some of these biopesticides on rats and humans have been reported. Awe and Oyetunji (2013) reported the toxicity of cassava leaf aqueous extracts on rats. Kadiri *et al*. (1999) found out that the traditional neem leaf-based medicines also used as biopesticide had acute toxic effects. Builders *et al.* (2012) administered graded doses of *Parkia biglobosa* stem bark methanolic and aqueous extracts orally to rats and found out that they were non-toxic.

Sherah *et al*. (2014) discovered the presence of substances that are cytotoxic enough to kill shrimp larvae in extracts of the seed-husk and stem-bark of *Parkia biglobosa*. The effectiveness of aqueous extract of *P. biglobosa* as a bio-insecticide in managing insect pests on okra plants had been reported by Fayinminnu *et al.* (2017). Therefore, the use of *P. biglobosa* aqueous extract as an insecticide on okra fruit vegetable necessitated the study of its toxicological effects. Information on the toxicity of residues of aqueous extract of *P. biglobosa* pod husk on rats is scanty. This study, therefore, aimed at assessing the sub-acute toxicity of residues of aqueous extract of *Parkia biglobosa* pod husk (PAPHE) on treated okra milled fruit feed on haematology, serum biochemistry and histology of kidney and liver organs of male wistar rats.

2. Materials and Methods

2.1. Preparation of aqueous extract of Parkia biglobosa pod husk

The pods of *Parkia biglobosa* were collected from Tede town in Atisbo Local Government Area, Oyo State (latitude $08^{o}34^{1}$N and longitude $003^{o}27^{1}$E). They were identified and authenticated at the herbarium of Forestry Research Institute of Nigeria, Jericho, Ibadan, Oyo State, Nigeria with voucher number 110449 (Fayinminnu *et al*., 2017). The extraction procedure of *Parkia biglobosa* pod husk was carried out at the Toxicology Research Laboratory of the Department of Crop Protection and Environmental Biology, University of Ibadan, Nigeria as reported by Fayinminnu *et al*. (2017). The pods of *P. biglobosa* were dehusked and the husks were air dried at room ambient temperature of 28.71± 1.77^{o}C and relative humidity of 77.64 ± 8.07% for two weeks. They were milled into powder using Rico MG '601' Grinder Mixer and sieved through a 2 mm sieve to remove the fibers (Fawole and Abikoye, 2002). Aqueous extract was obtained by soaking 250 grams of milled pod husk in 1250 ml of water (ratio 1:5) for 24 hours according to the method of Oshimagye *et al*. (2014). The extract was concentrated by placing it in water bath at low temperature (45- 50^{o}C) in order to determine its yield.

$$\% \text{ yield} = \frac{\text{weight of extract (grams)}}{\text{weight of plant material (grams).}} \times 100$$

The concentration of the stock solution of the biopesticide (aqueous extract of *P.biglobosa* pod husk (PAPHE)) was 20%; other concentrations were prepared from the stock solution by serial dilution. A 5% concentration was prepared by diluting 125 ml of the stock solution in 500 ml of water; 10% was prepared by diluting 250 ml of stock solution in 500 ml of water; 15% was prepared by diluting 375 ml of stock solution in 500 ml of water. The prepared extracts were labeled and stored in refrigerator at 20^{o}C for 24 hours prior to use to prevent putrefaction and degradation of phytochemicals present in them (Fayinminnu and Shiro, 2014).

2.1.1. Preparation of synthetic chemical pesticide

The synthetic chemical insecticide 'Scorpion' (Dimethoate 14.5% + Cypermethrin 5.5% (D+C)) was purchased from SARO Agrosciences Limited, Oluyole, Ibadan, Oyo State, Nigeria. Synthetic insecticide treatments were prepared as follows: 2.5 ml of dimethoate + cypermethrin in 500 ml (recommended dose) and 5 ml of dimethoate + cypermethrin in 500 ml of water (Dey *et al*., 2013).

2.2. Experimental Compounds

Qualitative and quantitative analyses of the following phytochemicals were performed on the milled *Parkia biglobosa* plant parts (leaves, bark, pod husk and seeds) at the Organic Chemistry (Pharmchem) Research Laboratory of the Faculty of Pharmacy, University of Ibadan and Femtop Analytical Laboratory, Idi-Ishin, Ibadan. According to the earlier work and report of Fayinminnu *et al*. (2017), the identified phytochemicals: Phenol, Flavonoid, Tannin, Saponin, Cardiac glycoside, Steroid, Terpenoid, Alkaloid and Antraquinone in *Parkia biglobosa* pod husk formed the basis for its potential biopesticide (insecticide) and baseline for this present study.

2.3. Field Study

This was carried out at the Crop Garden of the Department of Crop Protection and Environmental Biology (CPEB), University of Ibadan, Nigeria as reported earlier by Fayinminnu *et al*. (2017). The field seven treatments were: 2.5 and 5.0 ml D+C, 5, 10, 15, 20% PAPHE and control (0%). The gross area at the crop garden of CPEB was 22.3 m x 10.7 m with individual plot sizes of 0.9 m x 0.9 m and an alley of 2 m. Each of the seven treatments was replicated three times and laid out in a randomized complete block design.

Two seeds of okra (variety NHAe-47-4 purchased from National Horticulture Research Institute ((NIHORT), Idi-Ishin, Ibadan, Oyo State, Nigeria) were sown per stand at a depth of 2cm, spacing of 0.3m x 0.3m between each stand to obtain a plant population of 2651 plants ha^{-1}. Missing stands were supplied at one (1) week after sowing (WAS), while seedlings were later thinned to one (1) seedling per stand two (2) WAS. Watering was done manually on a daily basis. Regular weeding was carried out manually in order to prevent competition, infestation of pests and diseases and also to ensure maximum growth of the crops. The plants were sprayed with the aforementioned seven treatments weekly from two (2) WAS till twelve (12) WAS to control *Podagrica uniforma* and *sjostedti* (Flea beetles) using a 7.5 Litre knap sack sprayer.

At maturity, sprayed okra fruits were harvested at 9 to 12 weeks after sowing every week by plucking from each treated plot. Harvested sprayed okra fruits from each treatment were chopped into smaller pieces and air dried at an average room temperature of 28.71±1.77^{o}C and relative

humidity of 77.64 ± 8.07%, milled separately and stored in the refrigerator at 4°C for 24 hrs prior to use.

2.4. Experimental Animals

Thirty-two male wistar rats (*Ratus norvegicus*) weighing 80±20gm were obtained from the Central Animal House of the Department of Physiology, College of Medicine, University of Ibadan, Nigeria. Prior to the arrival of the wistar rats, the rat cages (wooden wire) were properly cleaned and disinfected at the Department of Pharmacognosy, College of Medicine, University of Ibadan. Cages were fitted with drinkers that could comfortably drop water when imbibed by rats and the feeders were properly placed to eliminate feed spillage. The animals were kept under and maintained at 27±2°C, with 12 hour light, 12 hour dark cycles, relative humidity of 70-80%.

The rats were divided into eight experimental groups (treatments) with four rats and were labeled T1, T2, T3, T4, T5, T6, T7 and T8 in each cage and were acclimatised for seven days before the commencement of the feeding treatments which lasted for 21 days. Locally grower mash feed (Standard Ratio Feed) and water was given to the rats during acclimatisation and treatment periods *ad libitum*. During the experiment, the experimental procedures were approved and conducted following the guidelines of University of Ibadan Ethical Committee which conforms to the Ethical use of Animals (Clarke *et al*., 1996).

2.5. Sub Chronic Toxicity of the Plant Extract and Synthetic Insecticide

2.5.1. Parkia biglobosa and Dimethoate + Cypermethin

The grouped experimental animals were tagged T1, T2, T3, T4, T5, T6, T7 and T8 of four rats per cage. They were administered: Standard Ration Feed (SRF) + treated okra milled feed of *Parkia biglobosa* pod husk extract (PPHE) at 5, 10, 15 and 20%, SRF+ Dimethoate 14.5% + Cypermethrin (D+C) at 2.5 and 5.0 ml and two controls (control group rats were fed with SRF (standard ration feed only) and untreated okra milled feed +SRF),

T1 – Group of rats fed on SRF (Standard Ration Feed) + untreated okra milled feed

T2 – Group of rats fed on SRF 93g +7g of okra milled feed sprayed with 2.5 ml of dimethoate + cypermethrin in 500 ml of water (recommended dose)

T3- Group of rats fed on SRF 93g +7g of okra milled feed sprayed with 5 ml of dimethoate + cypermethrin in 500 ml of water

T4- Group of rats fed on SRF 93g + 7g of okra milled feed sprayed with 20% extract of *Parkia biglobosa* pod husk extract

T5 – Group of rats fed on SRF 93g + 7g of okra milled feed sprayed with 15% extract of *Parkia biglobosa* pod husk extract

T6 – Group of rats fed on SRF 93g + 7g of okra milled feed sprayed with 10% extract of *Parkia biglobosa* pod husk extract

T7 – Group of rats fed on SRF 93g + 7g of okra milled feed sprayed with 5% extract of *Parkia biglobosa* pod husk extract

T8 – Group of Control rats (0%) (SRF only) 100g + 0g of okra milled feed

All groups were fed with okra treated milled feed + SRF and drinking water *ad libitum* was given for 21 days according to the treatments stated above everyday (from morning till night).

Weights of the animals were taken weekly and at the end of the experiment. Toxicity of the administered feed such as sluggishness, aggressiveness, weight gain / loss, convulsion, paralysis and mortality were observed on the experimental animals as daily routine as earlier reported by Fadina *et al*. (1999); Oshoke *et al*. (2016) and Fayinminnu *et al*. (2017).

2.6. Collection of Blood samples for hematology and Serum Biochemistry

2.6.1. Blood hematology and Serum Biochemistry Analyses

The termination of the experiment was done at 21 days; all the living wistar rats were sacrificed through cervical dislocation. Blood samples (2 ml) were collected by cardiac puncture into EDTAK3 bottles. They were arranged in heparinized capillary tubes and analyzed for hematological parameters according to the method of Schalm *et al*. (1975). The hematological parameters include Packed Cell Volume (PCV), Hemoglobin concentration (Hb), Red Blood Cell (RBC), White Blood Cell (WBC), Platelets (PLT), Lymphocytes (LYM), Neutrophiles (Neut), Monocytes (MON), Eosinophils (EOS), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC).

Blood samples for serum biochemical analyses were collected into plain bottles and centrifuged at 3000 revolutions per minute for 10minutes. The supernatant was analyzed for Total protein (TP) using Biuret method (Bradford, 1976), Albumin (ALB), Globulin (GLB), Total bilirubin (TB) concentrations were calculated according to Doumas *et al.* (1971). Alkaline Phosphatase (ALP), Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were determined using the method of Reitman and Frankel (1957), Glucose level, Creatinine and Urea.

2.7. Histopathological Examination

The experimental animals` abdomens were dissected immediately after blood collection to harvest organs of interest (liver and kidney). The tissues (livers and kidneys) were weighed and were preserved in 10% formalin in universal bottles for histopathological examination. They were then processed and stained with hematoxylin and eosin (H & E) stain for histopathology examination. For preparing the animal tissues for microscopic examination, histological procedures were followed in a stepwise protocol they include: Fixation, Dehydration, Clearing, Infiltration, Embedding, Blocking, Sectioning and Staining. Detailed microscopic examination was carried out on the organs of both control and treated groups.

The hematological and serum biochemistry analyses and histopathological examinations were carried out by qualified pathologists at the Clinical Pathology and Histopathology Laboratories of the Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria.

2.8. Statistical Analysis

Data were analyzed using analysis of variance (ANOVA) with Statistical Analysis System (SAS) software at 5% ($p<0.05$) level of significance. Means were

separated using Duncan Multiple Range Test (DMRT). Results were presented as mean ± standard error of the mean (SEM).

3. Results and Discussions

3.1. Behavioral Observations

The behavioral observation of the experimental animals during the study showed restlessness after each feeding to the treatments of *Parkia biglobosa* pod husk extract and dimethoate + cypermethrin treated okra. Restlessness, which is a sign of pesticide poisoning, was observed in animals fed okra treated with SRF + 5 ml of dimethoate + cypermethrin. This might be due to the inhibitory action of dimethoate (an organophosphate insecticide) on acetylcholinesterase leading to accumulation of acetylcholine at the nerve synapses (Chedi and Aliyu, 2010).

3.2. Toxic effects of treated okra with Parkia biglobosa pod husk extract (PAPHE) and dimethoate+ cypermethrin (D+C) on food intake of wistar rats

Result of treated okra feed intake of the experimental animals from this study varied across the three (3) weeks of feeding as shown in Figure 1. No significant differences ($p>0.05$) were observed at week one (1), although SRF+ 5 ml D+C (T3) had the highest value (79.7 g), followed by SRF only (T8) with 74.3g, the least value (52.5 g) was obtained from SRF+ 20% PAPHE (T4). Significant differences ($p>0.05$) in feed intake were not observed amongst the treatments at week two (2). However, SRF+5% PAPHE (T7) had highest value (74.3 g) followed by SRF+2.5 ml D+C (T2; 73.7 g), while treatment T1 (SRF+ untreated okra) had the least value of 42.9 g. The same trend as in week two was observed in week three with no significant differences among the treatments. Treatment SRF+ 5% PAPHE (T7) had the highest value (74.7 g), followed by SRF+ 2.5 ml D+C (T2; 69.4 g), while T1(SRF+ untreated okra) had the lowest value (50.8 g) though it increased compared to week two (2).

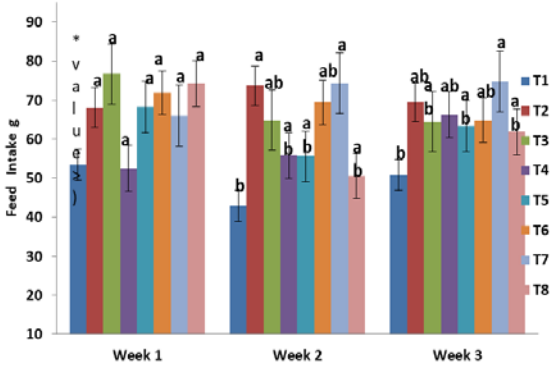


Figure 1: Toxic effects of treated okra with *Parkia biglobosa* pod husk extract (PAPHE) and dimethoate+ cypermethrin (D+C) on feed intake of wistar rats .T1= rats fed untreated okra + standard ration feed, T2 = rats fed okra treated with 2.5 ml of synthethic insecticide in 500 ml of water + standard ration feed, T3 = rats fed okra treated with 5 ml of synthetic insecticide in 500 ml of water + standard ration feed, T4 = rats fed okra treated with 20% PAPHE + standard ration feed, T5 = rats fed okra treated with 15% PAPHE + standard ration feed, T6 = rats fed okra treated with 10% PAPHE + standard ration feed, T7 = rats fed okra treated with 5% PAPHE + standard ration feed, T8 = control (rats fed with standard ration feed).

3.3. Toxic effects of (PAPHE) and dimethoate+ cypermethrin (D+C) on body weight of wistar rats

The toxic effect of varying doses of PAPHE and dimethoate + cypermethrin on mean body weights of wistar rats is presented in Figure 2. There were no significant differences in mean body weights among all the experimental animals at week zero (0) before the commencement of the treatments. Control animals (T8) had the highest mean weight (118.17g) which was not significantly different from treatments T1 (94.43g), T2 (97.83g), T4 (98.67g), T5 (95.5g) and T6 (98.6g) at the first week of treatment (week one). The mean weight of the control animals was significantly higher than that of treatments T3 and T7. Results shown at week two (2) revealed no significant differences in the mean weight of the animals. Treatment T6 had the highest mean weight (121.87g), closely followed by T7 (120.4g), T1 (118.47g), T5 (118.23g), T8 (118.1g), T2 (116.1), T4 (105.7g) and treatment T3 had the lowest (97.2 g) which was not significantly different. The body weight of the animals at week three showed that treatment T1 had the highest mean weight (136.87g) which was not significantly different from T2 (115.13g), T4 (113.23g), T5 (118.73g), T6 (112.73g), T7 (131.63g) and T8 (131.9g). However, this was significantly higher than treatment T3 (104.9g) which had the lowest weight among the treated animals.

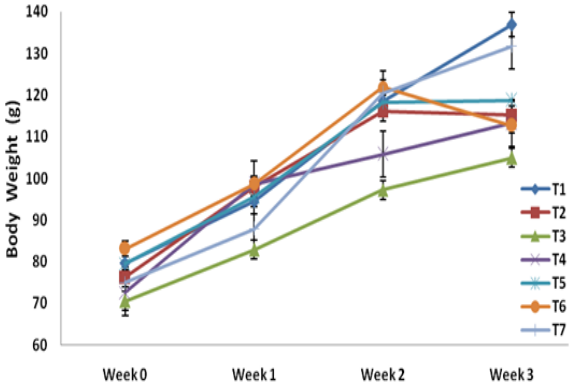


Figure 2: Toxic effects of treated okra with *Parkia biglobosa* pod husk extract (PAPHE) and dimethoate+ cypermethrin (D+C) on body weight of wistar rats **.**T1= rats fed untreated okra + standard ration feed, T2 = rats fed okra treated with 2.5 ml of synthethic insecticide in 500 ml of water + standard ration feed, T3 = rats fed okra treated with 5 ml of synthetic insecticide in 500 ml of water + standard ration feed, T4 = rats fed okra treated with 20% PAPHE + standard ration feed, T5 = rats fed okra treated with 15% PAPHE + standard ration feed, T6 = rats fed okra treated with 10% PAPHE + standard ration feed, T7 = rats fed okra treated with 5% PAPHE + standard ration feed, T8 = control (rats fed with standard ration feed)

The decrease in mean body weight of treated experimental animals was relatively non-significant in this study. This may be due to the rich diet they were fed with. Standard ration feed (SRF) was compounded according to the nutritional requirements of the rats (NebGuide, 2008) and mixed with milled okra which also contains nutrients required for proper growth (Sorapong, 2012) of the animals. This conformed to the findings of Builders *et al.* (2012) who observed no significant changes in the body weight of rats exposed to extracts of *Parkia biglobosa* stem bark.

3.4. a. Toxic Effects of Parkia biglobosa Pod Husk Extract (PAPHE) and dimethoate + cypermethrin treated okra on Hematological parameters of Wistar rats

Results presented in Table 1 showedHematological parameters: Packed cell volume (PCV), Hemoglobin concentration (Hb), Red blood cell count (RBC), White blood cell count (WBC), Platelet count (PLT) and Lymphocyte (LYM) of the male wistar rats. There were no significant differences ($p>0.05$) in PCV, HB, RBC, WBC and LYM across all the treated animals. Rats fed on okra treated with 15% PAPHE + SRF (treatment T5) had a significantly ($p<0.05$) higher platelet count (PLT) (129 $x10^3/\mu L$) over other treated animals.

Platelets are blood cells that circulate within the blood and bind together when they recognize damaged blood vessels. A higher number of platelets is referred to as Thrombocytosis. Thrombocytosis could be primary (when abnormal cells in the bone marrow cause an increase in platelets, but the reason is unknown) or secondary (abnormal cells in the bone marrow that may be caused by an ongoing condition or disease such as anaemia, cancer, inflammation or infection) (Williams, 2016). The 15% *Parkia biglobosa* aqueous pod husk extract (PAPHE) concentration increased the platelets of experimental animals; this could have been due to its ability to catalyze the bone marrow's activity of producing more platelets. Increased platelet counts could lead to increased platelet plug formation (Thrombosis). This could eventually lead to Thrombocythemia (restricted blood flow due to platelet formation), a condition that can cause severe ill health and death of animals. A lower platelet count was observed in animals fed okra treated with 20% (a higher) extract concentration. It may be deduced that 15% concentration of *P. biglobosa* aqueous pod husk extract is a threshold limit that causes a higher platelet count.

Table 1a. Toxic Effects of *Parkia biglobosa* Pod Husk Extract (PAPHE) and dimethoate + cypermethrin treated okra on Hematological parameters of Wistar rats

Treatments	PCV (%)	HB (g/dl)	RBC ($x10^3/\mu L$)	WBC ($x10^3/\mu L$)	PLT($x10^3/\mu L$)	LYM ($x10^3/\mu L$)
T1	38.0 ± 1.0^a	12.63 ± 0.34^a	6.35 ± 0.13^a	8.13 ± 1.31^a	113 ± 1.76^a	6.0 ± 1.20^a
T2	41.67 ± 1.76^a	13.50 ± 0.59^a	6.75 ± 0.34^a	6.13 ± 0.61^a	103 ± 13.3^a	4.08 ± 0.37^a
T3	45.33 ± 3.18^a	14.93 ± 1.14^a	7.50 ± 0.60^a	5.32 ± 0.86^a	115 ± 7.42^a	4.20 ± 0.53^a
T4	42.00 ± 1.73^a	13.70 ± 0.49^a	7.04 ± 0.35^a	3.90 ± 0.17^a	96.0 ± 6.7^a	2.67 ± 0.21^a
T5	47.33 ± 0.88^a	16.50 ± 0.95^a	7.62 ± 0.26^a	7.45 ± 0.32^a	219 ± 1.21^{ab}	5.27 ± 0.35^a
T6	41.33 ± 3.18^a	13.40 ± 1.06^a	6.64 ± 0.69^a	6.13 ± 1.60^a	103 ± 3.0^a	4.61 ± 1.26^a
T7	42.00 ± 1.53^a	13.60 ± 0.40^a	6.96 ± 0.35^a	5.70 ± 2.19^a	126 ± 29.1^a	4.23 ± 1.62^a
T8	45.17 ± 2.91^a	15.10 ± 0.89^a	7.54 ± 0.55^a	5.13 ± 1.26^a	117 ± 6.66^a	3.49 ± 0.93^a
	NS	NS	NS	NS		NS

Note: Values with the same superscripts (a) in the column indicate no significant difference from each other. $P>0.05$ means there is no significant difference, $P<0.05$ means there is significant difference at 5% level of probability using Duncan Multiple Range Test, NS= Not Significant.T1= rats fed untreated okra + standard ration feed, T2 = rats fed okra treated with 2.5 ml of synthethic insecticide in 500 ml of water + standard ration feed, T3 = rats fed okra treated with 5 ml of synthetic insecticide in 500 ml of water + standard ration feed, T4 = rats fed okra treated with 20% PAPHE + standard ration feed, T5 = rats fed okra treated with 15% PAPHE + standard ration feed, T6 = rats fed okra treated with 10% PAPHE + standard ration feed, T7 = rats fed okra treated with 5% PAPHE + standard ration feed, T8 = control (rats fed with standard ration feed).PVC= Packed cell volume, Hb= Hemoglobin concentration, RBC=Red blood cell count, WBC= White blood cell count, PLT =Platelet, LYM= Lymphocyte

b.Toxic Effects of Parkia biglobosa Pod Husk Extract (PAPHE) and dimethoate + cypermethrin treated okra on Hematological parameters of Wistar rats

The results shown in Table 1b revealed that rats fed on okra treated with 15% PAPHE + SRF (treatment T5) had a significantly higher (21.6 g/dL) Mean Corpuscular Hemoglobin (MCH). The other parameters (NEUT, MON, EOS, MCV and MCHC) across all other treatments were not significantly different ($p>0.05$).

Mean Corpuscular Hemoglobin (MCH) is the average amount of hemoglobin per red blood cell in a blood sample. Significantly higher MCH observed in experimental animals fed okra treated with 15% PAPHE is indicative of red blood cells with increased hemoglobin content. This might be suggestive of a macrocytic anemic condition (Ashaolu *et al*., 2011).

Table 1b: Toxic Effects of P*arkia biglobosa* aqueous pod husk extract (PAPHE) and dimethoate + cypermethrin treated okra on Hematological parameters of male wistar rats

Treatments	NEUT ($x10^3$)	MON ($x10^3$)	EOS ($x10^3$)	MCV (fl)	MCH(g/dL)	MCHC (pg)
T1	1.81 ± 0.31^a	0.16 ± 0.1^a	0.17 ± 0.04^a	59.80 ± 0.33^a	19.88 ± 0.12^a	33.25 ± 0.16^a
T2	1.78 ± 0.26^a	0.16 ± 0.1^a	0.12 ± 0.03^a	61.80 ± 0.48^a	20.02 ± 0.13^a	32.4 ± 0.05^a
T3	0.90 ± 0.26^a	0.13 ± 0.1^a	0.17 ± 0.02^a	60.63 ± 0.69^a	19.95 ± 0.24^a	32.91 ± 0.28^a
T4	1.04 ± 0.1^a	0.12 ± 0.1^a	0.08 ± 0.1^a	59.74 ± 0.75^a	19.50 ± 0.28^{ab}	32.63 ± 0.21^a
T5	1.60 ± 0.17^a	0.1 ± 0.0^a	0.17 ± 0.02^a	62.16 ± 1.18^a	21.61 ± 0.49^{abc}	34.81 ± 1.38^a
T6	1.31 ± 0.31^a	0.1 ± 0.0^a	0.12 ± 0.1^a	62.65 ± 1.98^a	20.30 ± 0.62^a	32.41 ± 0.13^a
T7	1.22 ± 0.48^a	0.16 ± 0.1^a	0.12 ± 0.1^a	60.40 ± 0.93^a	19.57 ± 0.42^{ab}	32.40 ± 0.30^a
T8	1.40 ± 0.26^a	0.15 ± 0.4^a	0.1 ± 0.03^a	60.65 ± 0.56^a	20.07 ± 0.32^a	33.09 ± 0.36^a
NS	NS	NS	NS			NS

Note: Values with the same superscripts (a, ab) in the column indicate no significant difference from each other. $P>0.05$ means there is no significant difference, $P<0.05$ means there is significant difference at 5% level of probability using Duncan Multiple Range Test, NS= Not Significant.T1= rats fed untreated okra + standard ration feed, T2 = rats fed okra treated with 2.5 ml of synthethic insecticide in 500 ml of water + standard ration feed, T3 = rats fed okra treated with 5 ml of synthetic insecticide in 500 ml of water + standard ration feed, T4 = rats fed okra treated with 20% PAPHE + standard ration feed, T5 = rats fed okra treated with 15% PAPHE + standard ration feed, T6 = rats fed okra treated with 10% PAPHE + standard ration feed, T7 = rats fed okra treated with 5% PAPHE + standard ration feed, T8 = control (rats

fed with standard ration feed).Neutrophils (NEUT), Monocytes (MON), Eosonophils (EOS), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC)

This present study showed that other hematological parameters such as Packed cell volume, Hemoglobin concentration, Red blood cell count, White blood cell count, lymphocytes, neutrophils, monocytes, eosinophils, mean corpuscular volume and mean corpuscular hemoglobin concentration had no significant differences in all treated experimental animals. This is similar to the findings of Builders *et al*. (2012).

3.5. Toxic Effects of Parkia biglobosa aqueous pod husk extract (PAPHE) and dimethoate + cypermethrin treated okra on Serum biochemical parameters of wistar rats

The serum biochemical parameters include Total protein (TP), Albumin (ALB), Globulin (GLOB), Albumin – Globulin (AG) ratio and Glucose (GLU) level (Table 2). The Total Protein of rats fed okra treated with 15% extract (PAPHE) + SRF (treatment T5) was significantly higher (8.7 g/dl) than all other treatments as shown on Table 2. The increase in serum Total Protein observed in animals administered treatment T5 (okra treated with 15% PAPHE + SRF) may have been due to increased release of tissue specific enzymes, intracellular proteins (Orhue *et al*., 2005) or acute phase proteins. Acute phase proteins are proteins whose concentration in the blood may increase as the body fights infection or some other inflammation.

Experimental animals fed on okra treated with 15% extract + SRF (T5) had a significantly higher albumin value (4.1 g/dl) than other animals. The Albumin of rats fed on untreated okra + SRF (T1) (2.4 g/dl), rats fed okra treated with SRF + 5 ml D+C (T3) (2.97 g/dl) and rats fed okra treated with 20% extract + SRF (T4) (3.07 g/dl) were significantly lower than that of rats fed on okra treated with SRF +2.5 ml D+C (T2) (3.40 g/dl) and rats fed okra treated with 10% extract + SRF (T6) (3.40 g/dl). There was no significant difference between rats fed okra treated with 5% extract + standard ration feed (T7) (3.27 g/dl) and the control T8 (3.47 g/dl). Albumin is produced entirely in the liver and is of great importance in regulating the flow of water between the plasma and tissue fluid by its effect on colloid osmotic pressure (Orhue *et al*., 2005). Damage in liver cells might have led to slightly higher blood albumin value as observed in animals to which treatment T5 was administered (Obaineh and Matthew, 2009).

The globulin of treatment T5 (4.57 g/dl) was significantly higher, while lower values of globulin were observed from treatments T2 (3.27 g/dl) T4 (3.90 g/dl), T6 (3.83 g/dl) and control (3.93 g/dl) with no significant differences. Slightly higher globulin value also observed in animals administered treatment T5 might have been due to enhanced antibody secretion in response to infection or allergy (Orhue *et al*., 2005).

Table 2. Toxic Effects of *Parkia biglobosa* aqueous pod husk extract (PAPHE) and dimethoate + cypermethrin treated okra on serum biochemical parameters of Wistar rats

Treatments	TP (g/dl)	ALB (g/dl)	GLOB (g/dl)	A.G Ratio	GLUC (mg/L)
T1	6.60±0.06^{a}	2.43±0.19^{a}	4.17±0.15^{a}	0.57±0.07^{a}	122±4.91^{a}
T2	7.17±0.12^{a}	3.40±0.15^{b}	3.77±0.07^{ab}	0.87±0.07^{a}	119±3.18^{a}
T3	7.03±0.13^{a}	2.97±0.22^{a}	4.07±0.09^{a}	0.67±0.07^{a}	122±2.91^{a}
T4	6.97±0.32^{a}	3.07±0.29^{a}	3.90±0.06^{ab}	0.77±0.09^{a}	130±3.7^{a}
T5	8.67±0.09^{b}	4.10±0.06^{abc}	4.57±0.09^{abc}	0.87±0.03^{a}	117±1.76^{a}
T6	7.23±0.07^{a}	3.40±0.21^{b}	3.83±0.15^{ab}	0.80±0.06^{a}	131±4.04^{a}
T7	7.27±0.15^{a}	3.27±0.07^{ab}	4.00±0.10^{a}	0.77±0.03^{a}	121±3.51^{a}
T8	7.40±0.15^{ab}	3.47±0.12^{ab}	3.93±0.13^{ab}	0.83±0.03^{a}	115±1.33^{a}
				NS	NS

Note: P>0.05 means there is no significant difference, P<0.05 means there is significant difference at 5% level of probability using Duncan Multiple Range Test, NS = Not Significant

T1= rats fed untreated okra + standard ration feed, T2 = rats fed okra treated with 2.5 ml of synthethic insecticide in 500 ml of water + standard ration feed, T3 = rats fed okra treated with 5 ml of synthetic insecticide in 500 ml of water + standard ration feed, T4 = rats fed okra treated with 20% PAPHE + standard ration feed, T5 = rats fed okra treated with 15% PAPHE + standard ration feed, T6 = rats fed okra treated with 10% PAPHE + standard ration feed, T7 = rats fed okra treated with 5% PAPHE + standard ration feed, T8 = control (rats fed with standard ration feed) TP= Total protein, ALB =Albumin, GLOB =Globulin, AG= Globulin ratio, GLU = Glucose level

3.6. Toxic Effects of Parkia biglobosa aqueous pod husk extract (PAPHE) and dimethoate + cypermethrin on enzyme markers of kidney function of male wistar rats

The results as shown on Table 3 revealed no significant differences (p>0.05) in TB, ALP, ALT and Urea across all treated experimental animals. The creatinine level in treatment T5 was significantly higher (1.13 mg/dL) than other experimental animals. Creatinine is excreted from the body in the urine via the kidneys. As a result, creatinine measurement is used almost exclusively in the assessment of kidney function. Creatinine level was higher in animals administered treatment T5 when compared to other treatments. Elevation of creatinine is indicative of under-excretion, suggesting kidney impairment (Oyebanji *et al*., 2013).

A significant similar trend was also observed in AST level (45.67 U/L). Aspartate Amino Transferase (AST) a serum liver enzyme had a slightly higher value in animals administered treatment T5 when compared to the control and other treatments. This might be indicative of liver cell damage (Aniagu *et al*., 2005).

Table 3: Toxic Effects of *Parkia biglobosa* aqueous pod husk extract (PAPHE) and dimethoate + cypermethrin on enzyme markers of kidney and liver functions of male wistar rats

Treatments	TB (mg/dL)	AST (U/I)	ALT (U/I)	ALP (U/I)	UREA (mg/dL)	CREAT (mg/dL)
T1	0.3±0.1^{a}	43.0±1.53^{a}	31.33±0.88^{a}	112±2.52^{a}	15.73±0.44^{a}	0.67±0.03^{ab}
T2	0.23±0.09^{a}	39.67±0.88^{a}	27.33±1.45^{a}	111±7.84^{a}	16.33±0.69^{a}	0.73±0.03^{ab}
T3	0.23±0.09^{a}	43.33±2.03^{a}	30.67±1.20^{a}	111±4.67^{a}	14.77±0.58^{a}	0.67±0.03^{ab}
T4	0.33±0.03^{a}	37.0±0.58^{a}	25.33±0.33^{a}	111±3.93^{a}	15.30±0.44^{a}	0.733±0.03^{ab}
T5	0.27±0.03^{a}	45.67±0.88^{ab}	33.33±0.33^{a}	118±7.31^{a}	16.73±0.79^{a}	1.13±007^{abc}
T6	0.17±0.03^{a}	42.67±1.45^{a}	31.0±1.53^{a}	118±1.0^{a}	14.30±1.11^{a}	0.80±0.0^{ab}
T7	0.23±0.88^{a}	40.33±0.88^{a}	29.0±2.0^{a}	130±1.20^{a}	16.77±0.32^{a}	0.73±0.03^{ab}
T8	0.23±0.88^{a}	37.0±1.16^{a}	26.0±0.58^{a}	121±6.25^{a}	16.40±0.58^{a}	0.67±0.07^{ab}
	NS		NS	NS	NS	

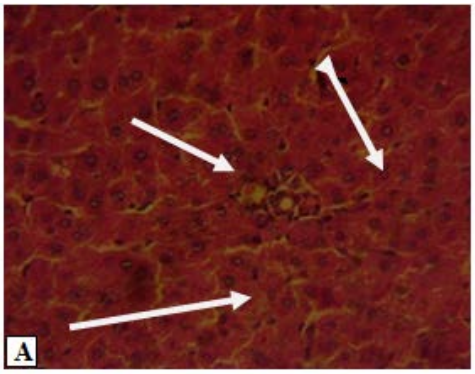
Note: Values with the same superscripts (a, ab) in the column indicate no significant difference from each other. P>0.05 means there is no significant difference, P<0.05 means there is significant difference at 5% level of probability using Duncan Multiple Range Test, NS= Not Significant.T1= rats fed untreated okra + standard ration feed, T2 = rats fed okra treated with 2.5 ml of synthethic insecticide in 500 ml of water + standard ration feed, T3 = rats fed okra treated with 5 ml of synthetic insecticide in 500 ml of water + standard ration feed, T4 = rats fed okra treated with 20% PAPHE + standard ration feed, T5 = rats fed okra treated with 15% PAPHE + standard ration feed, T6 = rats fed okra treated with 10% PAPHE + standard ration feed, T7 = rats fed okra treated with 5% PAPHE + standard ration feed, T8 = control (rats fed with standard ration feed).TP= Total protein, ALB =Albumin, GLOB =Globulin, AG= Globulin ratio, GLU = Glucose level

3.7. Toxic effects of Parkia biglobosa Pod Husk Extract and dimethoate + cypermethrin treated okra on Histological organs (liver and kidney) of male wistar rats

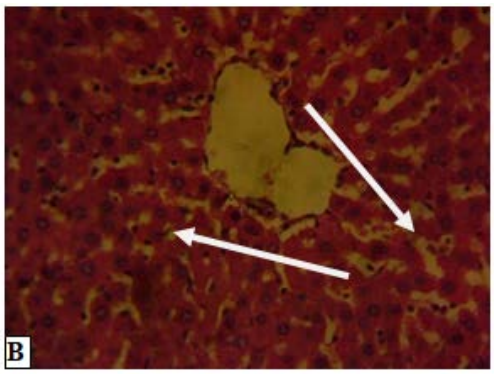
Photomicrographs showing sections of organs of interest (liver and kidney) of the treated rats are presented in plates 1 and 2. Parenchymal cells of the liver (i.e. the hepatocytes) and kidney (i.e. glomerulus and tubular epithelial cells) were normal in treatments T1: rats fed untreated okra + standard ration feed (control), T7: rats fed okra treated with 5% PAPHE + standard ration feed, and T8: control (rats fed with standard ration feed only). Lesions were observed in the livers of the following treatments: T2: rats fed okra treated with 2.5 ml of dimethoate + cypermethrin + standard ration feed, T3: rats fed okra treated with 5 ml of dimethoate + cypermethrin + standard ration feed, T4: rats fed okra treated with 20% PAPHE + standard ration feed, T5: rats fed okra treated with 15% PAPHE + standard ration feed, T6: rats fed okra treated with 10% PAPHE + standard ration feed. Similarly, lesions were also observed in the kidneys of treatments T4 and T6.

Experimental rats fed on T1 showed normal hepatocytes, glomerulus and tubules in their liver and kidney, respectively (control). Moderate centrilobular vacuolar degeneration and atrophy of hepatocytes were observed in liver, while kidney had normal histological structures in T2 treatment. Examination showed diffuse atrophy of hepatic cords and Kupffer cells hyperplasia in the liver of T3 with normal histological structures in the kidney. Observations in T4 showed mild random degeneration of hepatocytes in liver, while the kidney revealed diffuse vacuolar degeneration of medullary tubular epithelial cells (T1-T4 shown in plate 1).

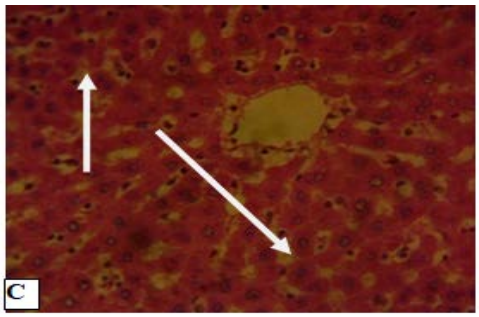
Diffuse degeneration of hepatocytes; Hepatocellular necrosis and infiltrate of inflammatory cells were shown in liver of T5, while kidney showed normal histological structures. Experimental rats fed on T6 treatment showed liver with marked random degeneration and necrosis of hepatocytes, while the kidney had coagulation necrosis of tubular epithelial cells. Normal histological structures were observed in both liver and kidney of T7 as well as in T8 (control) (T5-T8 as shown in plate 2).



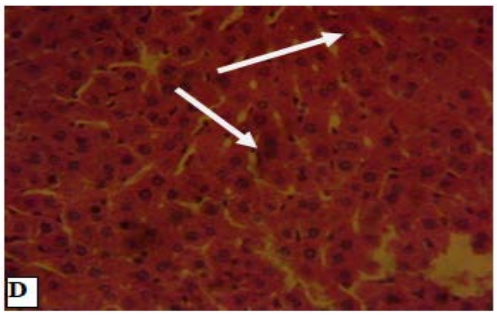
Arrows showing Normal hepatocytes



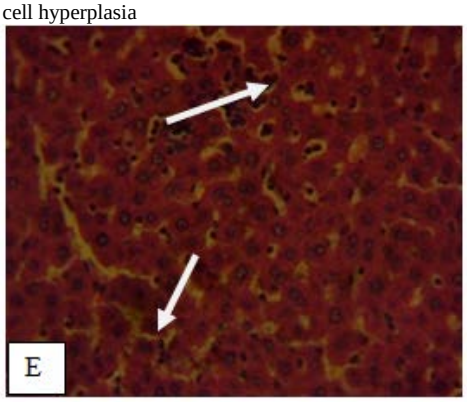
Arrows showing Moderate centrilobular vacuolar degeneration and atrophy of hepatocytes



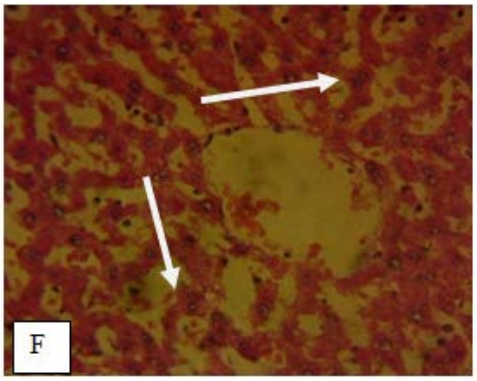
Arrows showing Diffuse atrophy of hepatic Kupffer cell hyperplasia



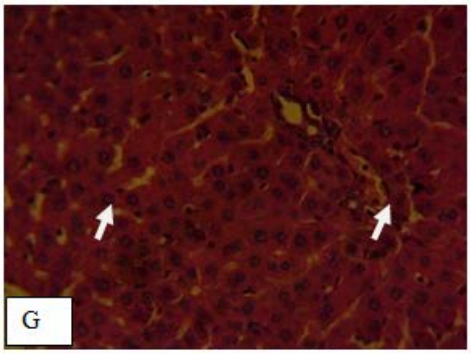
Arrows showing Mild random degeneration of cord and hepatocytes



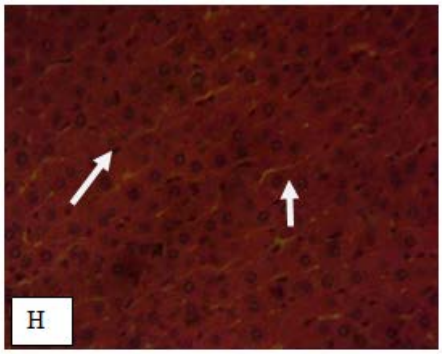
Arrows showing Focus of hepatocellular infilterate of inflammatory cells



Arrows showing Random degeneration and necrosis of hepatocyte

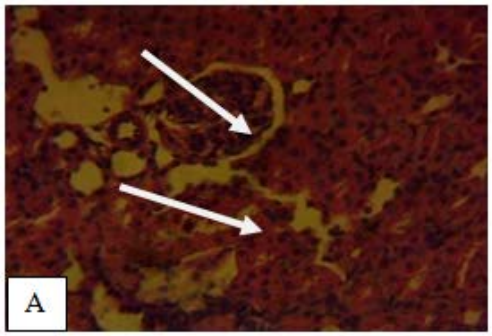


Normal histological structures

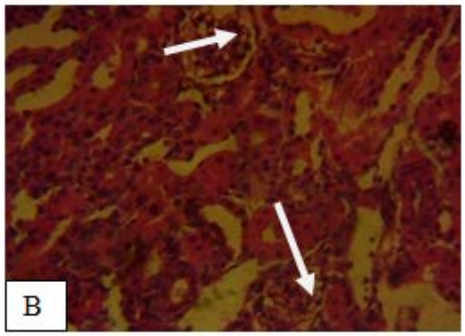


Normal histological structures

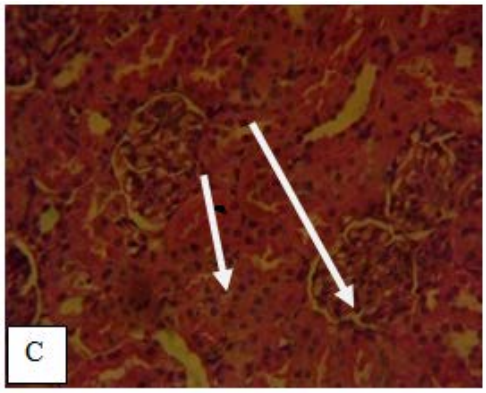
Plate 1: Photomicrograph of section of rat liver fed with okra treated with *Parkia biglobosa* and dimethoate + cypermethrin treatments.**A - T1**: Liver of rat fed with untreated okra + standard ration feed, **B – T2**: Liver of rat fed okra treated with 2.5 ml of dimethoate + cypermethrin + standard ration feed (SRF) ,**C- T3**: Liver of rat fed okra treated with 5 ml of dimethoate + cypermethrin + SRF,**D** – T4: Liver of rat fed okra treated with 20% extract (PAPHE) + SRF **,**(H & E Magnification X 400). **E – T5**: Liver of rat fed okra treated with 15% PAPHE + SRF.**F - T6:** Liver of rat fed okra treated with 10% PAPHE + SRF.**G – T7**: Liver of rat fed okra treated with 5% PAPHE + SRF. **H – T8**: Liver of rats fed with SRF only (control) .(H & E Magnification X 400)



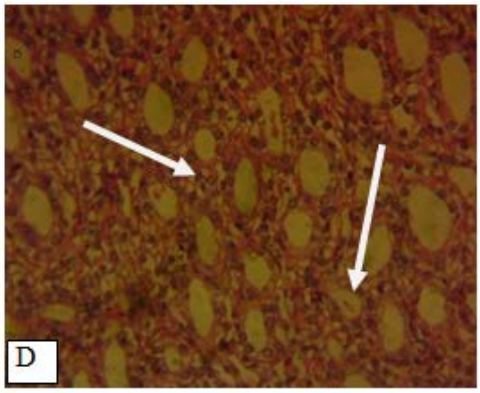
Arrows showing Normal glomerulus and tubules



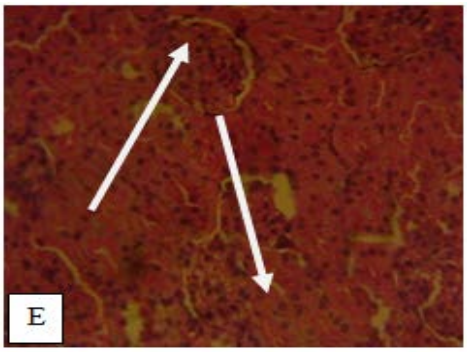
Arrows showing Normal histological structures



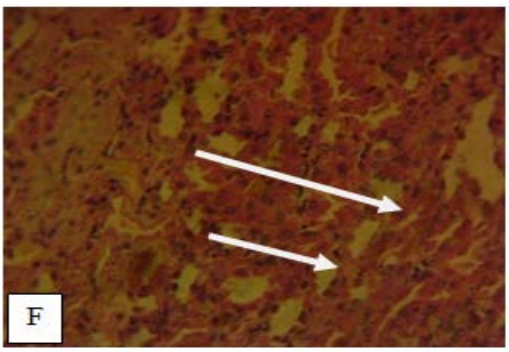
Arrows showing Normal histological structures of medullary



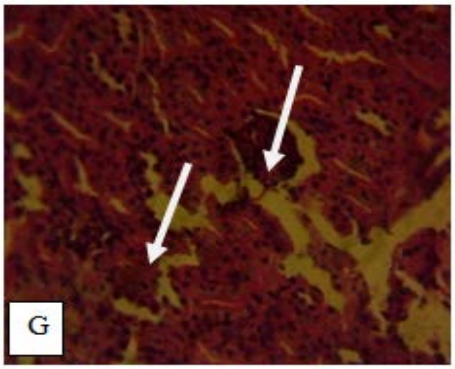
Arrows showing Diffuse vacuolar degeneration tubular epithelial cells



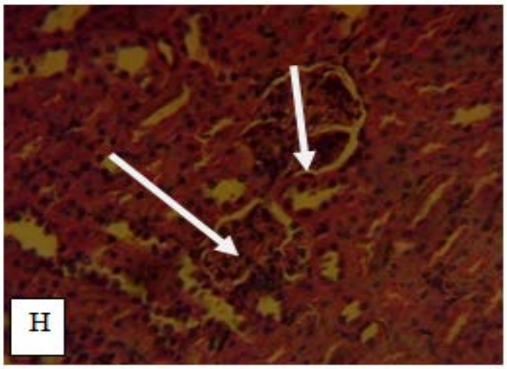
Arrows showing Normal histological structures



Arrows showing Coagulation necrosis of tubular epithelial cells



Arrows showing Normal histological structures



Arrows showing Normal histological structures

Plate 2: Photomicrograph of section of rat kidneys fed with okra treated with *Parkia biglobosa* and dimethoate + cypermethrin treatments **A - T1**: Kidney of rat fed with untreated okra + Standard Ration Feed, **B – T2**: Kidney of rat fed okra treated with Standard Ration Feed + 2.5 ml of D+C ,**C- T3**: Kidney of rat fed okra treated with Standard Ration Feed + 5 ml of D+C ,**D – T4**: Kidney of rat fed okra treated with 20% PAPHE + Standard Ration Feed**, E – T5**: Kidney of rat fed okra treated with 15% PAPHE + Standard Ration Feed**, F - T6:** Kidney of rat fed okra treated with 10% PAPHE + Standard Ration Feed**, G – T7:** Kidney of rat fed okra treated with 5% PAPHE + Standard Ration Feed**, H – T8:** Kidney of rats fed with Standard Ration Feed only (control)
(H & E Magnification X 400)

Histopathology analyses conducted in this study revealed lesions such as vacuolar degeneration, atrophy, necrosis of hepatocytes and kupffer cells in photomicrographs of liver of the following treatments: rats fed okra treated with SRF+ 2.5 ml of Dimethoate + Cypermethrin (T2), rats fed okra treated with SRF+ 5 ml of Dimethoate + Cypermethrin (T3), rats fed okra treated with 20% PAPHE + SRF (T4), rats fed okra treated with 15% PAPHE + SRF (T5) and rats fed okra treated with 10% PAPHE + SRF (T6). This might be indicative of liver cell damage or hepatotoxicity (Gregorio, 2001) as the liver is the primary site of metabolism and detoxification. The plant extract (*Parkia biglobosa* aqueous pod husk extract) and synthetic insecticide (Dimethoate + Cypermethrin) did not really alter the morphology of the kidney, as lesions were observed in photomicrographs of kidney of animals administered treatments T4 and T6 only. This might be indicative of renal toxicity. Lesions were not observed both in the liver and kidney organs of animals fed with untreated okra + SRF (treatment T1), okra treated with 5% extract + SRF (treatment T7) and standard ration feed (SRF) only (treatment T8/ control).

Vacuolar degeneration is a reversible cell injury (Seely and Brix, 2016); the liver of experimental animals in which vacuolar degeneration was observed could revert back to normal if the treatment is stopped. Atrophy refers to a reduction in cell size; on the other hand, necrosis (also a type of lesion) is an irreversible reaction to injury otherwise called cell death (Syntichaki and Tavernarakis, 2002). If treatments of the experimental animals with the toxicants continue for a longer period or at higher concentrations, death of the cells may occur.

4. Conclusion

The variations observed in the levels of hematology and serum biochemical parameters were results of different concentrations of *Parkia biglobosa* aqueous pod husk extract (PAPHE) and synthetic insecticide (Dimethoate + Cypermethrin) used in the treatments relative to the control. The toxic effects of PAPHE and dimethoate + cypermethrin on the organs of fed okra wistar rats may be due to any or a combination of all the phytochemicals present in the aqueous pod husk extract.

This study revealed that rats exposed to feed containing untreated okra + standard ration feed, standard ration feed only and okra treated with 5% PAPHE + standard ration feed were free from organ damages. As such, the aqueous pod husk extract of *Parkia biglobosa* appears to be safe for consumption at 5% concentration in agricultural sustainability, food quality and safety.

Acknowledgments

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