Toxicological Effects Of Aqueous Extract Of *Anogeissus Leiocarpus* Leaf, *Carica Papaya* Leaf, And *Mangifera Indica* Stem Bark (A Herbal Product Used Against Typhoid Fever) On Albino Rats.

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Abstract: The effects of various concentrations of aqueous extract of *Anogeissus leoicarpus (marke)* leaf, *Carica papaya (pawpaw)* leaf and *Mangifera indica (mango) stem* barks on some biochemical parameters, haematological parameters and histopathology were investigated in albino rats. The LD_{50} could not be calculated as no death was recorded even at a high dose of 5000 mg/kg. Phytochemical analysis of the plants' materials showed the presence of resins, alkaloids, saponins, tannins, glycosides and flavonoids in marke. No glycosides in mango and no tannins in pawpaw. The biochemical and haematological values revealed no statistical significant difference ($p < 0.05$) between the control groups and the groups fed with the crude plants' extracts. The results, therefore, strongly suggest that these aqueous plants' extracts are safe for oral consumption as they appear not to be hepatotoxic in rats, rather the animals increased in weight and had improved haematological values. The findings are of nutritional, clinical and veterinary relevance considering the diverse applications of these plants in most populations.
[V.N Chidozie and G.I Adoga. **Toxicological Effects Of Aqueous Extract Of *Anogeissus Leiocarpus* Leaf, *Carica Papaya* Leaf, And *Mangifera Indica* Stem Bark (A Herbal Product Used Against Typhoid Fever) On Albino Rats.** *Cancer Biology* 2014;4(4):26-34]. (ISSN: 2150-1041). http://www.cancerbio.net. 4

Key words: *Anogeissus leoicarpus*, *Carica papaya, Mangifera indica,* Biochemical, Haematological, Histopathology, LD50.

Introduction

All over the world, medicinal plants are gaining very high popularity in the treatment and management of diverse kinds of diseases because of their professed and proven efficacy. Also many people are gaining more confidence in the use of medicinal plants (Omonkhua and Onoagbe 2008) because microorganisms are developing resistance to many orthodox drugs coupled with the many adverse side effects of the orthodox drugs. Moreover in developing countries medicinal plants are more accessible and affordable. In Nigeria, West Africa, a survey of forest plants used in the traditional treatment of typhoid fever was conducted in Chikun local government area of Kaduna state by Faleyimu *et al.,* (2010). The phyto medication products involve a composition of two or more plants. One product involves the combination of *Anogeissus leoicarpus (marke)* leaf, *Carica papaya (pawpaw)* leaf and *Mangifera indica (mango) stem* bark. This combination is cooked with water. Some of which is used for bathing the patient while some is drunk by the patient. These plants used in combination are said to be very effective in the treatment of typhoid fever. The combination of these plants, as claimed by the herbalists, totally gets rid of typhoid fever within a space of one to two weeks. Care must be taken not to use plants or their extracts that could cause toxic side effects in the body either on short or long term use. For this reason, there is need to carry out scientific researches of the biochemical and toxicological effects of these medicinal plant.

Typhoid fever is a systemic infection with the bacterium *salmonella enteric serotype typhi*. This disease occurs mostly in developing countries like Nigeria where sanitary conditions are very poor. It is estimated that there is at least sixteen million new cases of typhoid fever each year, with six hundred thousand deaths (Ivanoff, *et al.,* 1995). Typhoid fever is usually contracted by ingestion of food or water contaminated by the faeces or urine of carriers excreting *Salmonella typhi*. The treatment of typhoid fever with chloramphenicol in 1948, transformed a severe, debilitating, and often fatal disease into a readily treatable condition (Woodward *et al.,* 1948). The emergence of resistance to chloramphenicol and other antimicrobial agents has been a major setback (Mizra *et al.,* 1998). We now face the very real prospect that untreatable typhoid fever will reemerge (Parry *et al.,* 2002). Hence the more reason why other types of treatment like the use of herbs should be greatly explored.

Anogeissus leiocarpus is a fodder tree occurring in most of savanna areas from the driest region to the borders of forest zones (Ibrahim *et al.,* 1997). It belongs to the family combrataceae.). It is commonly called the African Birch. In Nigeria, it is known as Otra in Idoma, Marke (or kwankila) in Hausa, Atara in Ibo and Orin-odan in Yoruba (Agaie and Amali,

2007). *A leiocarpus* has numerous medicinal applications all over Africa (Adigun *et al.,* 2000). In traditional medicine its infusion and decoction is used as cough medicine, the powdered root is applied to wounds and ulcers while powdered bark is rubbed on gums to reduce toothache (Ibrahim *et al.,* 1997). The decoction is used as vermifuge and for fumigation while leprotic, laxative and antihelmintic properties of the leaf extract have also been reported in man and animals (Burkill, 1985, Onyeyili, 2000). A leiocarpus is also used as an emulsifier, treatment for diarrhea, syphilis chancres, stimulant, aphrodisiac and tanicide for horses and donkeys (Adigun *et al.,* 2002). This plant has been shown to be active as antimicrobial agent against gram positive and gram negative bacteria (Adeleye *et al.,* 2003; Maichido and Ado 1999), antimycobacterial activity (Malcolm and Sofowora, 1969; Johnbull and Abdu, 2006; Uba *et al.,* 2003), trypanacidal activity (Atawodi *et al.,* 2003) and demonstrated activity against *Candida albicans* (Chaabi *et al.,* 2006; Sanogo *et al.,* 1997, Sanogo 2005). Several secondary metabolites such as tannins, saponins, alkaloids and flavonoids are reported to be present in the root, bark and leaf extracts of the plant (Burkill, 1985; Ibrahim *et al.,* 1997). Some of these metabolites have been shown to possess toxic potentials (Dicko and Sikena 1991).

Carica papaya is a native to the tropics of the Americas. It is distributed throughout Asia and Africa (Afolayan, 2003). It belongs to the family caricaceae. It has the following common names; pawpaw tree, papaya and papayer. The leaf poultice is used for nervous pains and elephantoid growths. The leaf is smoked for asthma relief in various remote areas (Reed, 1976). The aqueous leaf extract showed pronounced inhibition demonstrating a high activity against the test bacteria (Anibijuwon and Udeze 2009). The young leaves and to a lesser extent other parts of the plant contain carpain, an active bitter alkaloid which has a depressing action on the heart. (Rao and Agarwal, 2000).Papaya seed extract has antibacterial activity against Escherichia Coli, *Staphyloccus aureus* and *Salmonella typhi* (Yismaw *et al.,* 2008).Preclinical phytochemical analyses showed that the leaf extracts contain alkaloids, tannins, saponins, glycosides and phenols (Anbijuwon and Udeze, 2009).Papaya contains many biochemically active compounds. Two important compounds are chymopapain and papain which are supposed to aid digestion. Papain is also used in the treatment of arthritis (Anibijuwon and Udeze, 2009).

Mangifera indica a large ever green tree is native to Asia. Now it is completely naturalized in many parts of the tropics and subtropics (Ross, 1999). It is commonly called mango. It belongs to the family anacardiaceae. *Mangifera indica* is used against asthma, cough, diarrhea, dysentery, jaundice pains and malaria (Agoha 1981, Madunagu *et al.,* 1990). In all the region of *Mangifera indica* distribution, one of the main organs used is the bark. Based on ethnopharmacological knowledge, a standardized aqueous extract of the plant's stem bark has been developed in Cuba. This extract is proposed as both a nutritional supplement (antioxidant) and an anti-inflammatory, analgesic and immunomodulatory treatment to prevent disease progress or increase the patient's quality of life in gastric and dermatological disorders, Aids, cancer and asthma (Nuñez-selles, 2005). Mangiferin is one of the bioactive compounds that abound in mango and it is found to be antimicrobial (Zhu *et al.,* 1993; Zheng *et al.,* 1990 ; Guha *et al.,* 1996), antibacterial, antifungal (Stoilova *et al.,* 2005) and antiparasitic activities (Perrucci *et al.,* 2006). El Mahmood (2009) found that the crude extract of mango stem bark has alkaloids, phenols, tannins, saponins and cardiac glycosides.

Materials And Methods

Chemicals and Reagents

All laboratory reagents as far as possible were of analar grade.

Equipment

Blood cell counter BC-2800 Vet, Flame photometre, Spectrophotometre (Optima sp-300), Shandom automatic tissue processor, hot plate, Microscope, Mettler weighing balance, Ohaus Harvard trip balance, Hot air oven, Rotary evaporator.

Plant Material

Leaves of *Anogeissus leiocarpus, Carica papaya* and bark from the stem of *Mangifera indica* trees were freshly collected from Vom in Jos south L.G.A, and were identified at the Federal Department of Forestry Jos.

Experimental Animals

Albino rats of both sexes weighing 91g-180g were purchased from the small animal house of federal college of Verterinary and Medical Laboratory Technology, N.V.R.I, Vom and were maintained on pelleted feeds obtained from Dagwom farms.N.V.R.I, Vom

Methods

Hot Water Extraction of the Crude Plants' Materials

The leaves and the barks of the above mentioned plants were washed with clean water to get rid of dust and dirt. The leaves were air dried to get rid of the water droplets. The leaves were dried in an oven at 60^0C for five days. The barks were also oven dried at

60^0C for seven days. The dried plant materials were pulverized into coarse powder in a mortar with a pestle. They were separately, ground into fine powder with an electric blender. 100g of each plant sample were extracted separately in hot water. The solutions were filtered and the filterate evaporated off under reduced pressure in a rotary evaporator to obtain the crude extract.

Phytochemical Screening

The extracts were subjected to phytochemical screening to detect the presence of the following secondary metabolites; Resins, Alkaloids, Saponins, Tannins, Glycosides and Flavonoids following standard procedures (Trease and Evans, 1989).

Reconstitution of the Crude Extract Preparation

The crude extracts of the different plants were separately reconstituted in sterile distilled water to get the following concentrations: 10mg/kg, 100mg/kg, 1000mg/kg, 1600mg/kg, 2900mg/kg and 5000mg/kg body weight of the animals. Also10g of the three plants' crude extract were combined and reconstituted in sterile distilled water and concentrations of 10mg/kg – 5000mg/kg prepared as earlier outlined.

Acute Toxicity Determination

The acute toxicity studies were carried out as follows according to Lorke (1983) with slight modification. The acute toxicity was performed in two stages. Lorke's method is based on the assumption that the chemical substances under investigation are completely unknown and the investigation is to be carried out using a minimum number of experimental animals. The determination of the appropriate dosage range of the acute toxicity is achieved by giving orally, widely differing doses to the animals. E.g. 10mg/kg, 100mg/kg and 1000mg/kg body weight of the animals. The results show whether the chemical substance is very toxic, toxic, less toxic, slightly toxic or not toxic. In this initial investigation the range of the doses producing the toxic effects is established. Based on the results from stage 1 further high doses are administered to calculate the LD_{50}. The LD_{50} is calculated as the geometric mean of the doses for which 0/1 and 1/1 animals died. For example, at 2900mg/kg 0% animal died and at 5000mg/kg 100% of the animals died. The LD_{50} is the geometric mean of 2900 and 5000 which is 3800mg/kg.

A total of 16 animals are used for each plant extract. 9 animals for the 1st stage, 3 animals as the control, and four animals for the 2nd stage.

The animals were kept in the experimental house for five days for adaptation. Food and water were given *ad libitum*. The animals were given numbers using the conventional method of numbering animals. They were individually weighed using Ohaus Harvard trip balance. Food and water were withdrawn from them the night before the commencement of the experiment.

1st STAGE

The animals were divided into 3 groups of 3 animals per group. The 4th group has 3 animals and serves as the control. Group 1 was given the plant extract of 10mg/kg body weight of the animal, group 2 was given 100mg/kg and group 3 was given 1000mg/kg. Group 4 was given a volume of distilled water in commensuration with their body weights. The animals were observed frequently on the day of treatment until the office closing hour of 4pm. The animals were monitored to see if the plant extracts produced the effect of dermal or oral irritations as would be manifested as rubbing of mouth, stretching of limbs and scratching. Other symptoms of toxicity like difficulty in breathing, disinclination to move, or eat, sleepiness and death were observed for. On the first day of the experiment, the animals were monitored in hours and subsequently (for 14 days) the animals were monitored on daily basis. Also the animals were weighed before the commencement of the experiment and on the day of the termination of the experiment. Weight gain or otherwise was recorded. After 14 days the animals were anaesthetized with chloroform and the blood collected through direct heart puncture. 2mls were added into E.D.T.A anticoagulant bottles and 4mls into plane centrifuge tubes. The blood collected into anticoagulant bottles were used for haematological analysis while the serum was collected after centrifugation at 3000r.p.m for 5minutes for biochemical analysis. The animals were thereafter sacrificed and autopsied and examined macroscopically for any pathological changes. Samples of the liver, and kidneys were also collected into 10% formalin for histological studies.

2nd STAGE

The animals were divided into 4 groups of one animal per group. Group 1 was given a dose of 1600mg/kg body weight, group 2 was given 2900mg/kg body weight, group 3 was given 5000mg/kg and group 4 distilled water in a volume calculated from the body weight of the animal. Observations of the animals were carried out as in the 1st stage. Also the entire steps of the 1st stage were repeated until the experiment was terminated on the 14th day and LD_{50} calculated.

Method for Haematological Analyses

The blood collected (in EDTA as anticoagulant) was analyzed for total white blood cells (WBC), lymphocytes (LYMPH), monocytes (MONO), granulocytes (GRAN), red blood cells (RBC),

haemoglobin (HGB), haematocrit (HCT), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), red cell distribution width (RDW), platelets (PLT), mean platelet volume (MPV), platelet distribution width (PDW), and packed platelet volume (PCT) using fully automated blood cell counter BC-2800 Vet.

Methods for Biochemical Analyses

The serum collected from the clotted blood was analyzed using a flame photometre and spectrophotometre at the appropriate wavelengths using the appropriate reagents. Alkaline phosphatase activities was determined using the method as described by King Armstrong (1964). Serum albumin concentration was determined using the bromocresol green method. Aspartate amino transferase and alanine amino transferase were determined by the method of Reitman and Frankel (1957). Total protein content of the samples was assayed by the biuret method (Plummer, 1978). Sodium and Potassium ions content in the serum were measured using the flame photometer (Bassir, 1971). The procedure of Tietz *et al* (1994) was used to determine serum creatinine concentration while the serum urea concentration was determined by diacetyl monoxime method. The method of Malloy and Evelyn (1932) was employed to determine the serum bilirubin concentration of the samples.

Method for Histological Analysis

The samples of liver, kidney and heart collected separately into 10% formalin were processed using Shandom automatic tissue processor. The tissues sectioned into 5 micrometer sizes were attached to glass slides by water bath method. The section was allowed to dry on a hot plate. Harris haematoxylin and eosin stain was used to demonstrate the general tissue structure and viewed under a microscope.

Results

Phytochemical screening of the various plant extracts

The phytochemical screening of the aqueous crude extract of pawpaw leaf, marke leaf and mango stem bark shows that all the extracts contain resin, alkaloids, saponins and flavonoids, only marke and mango contain tannin and glycosides are seen only in marke and pawpaw (Table 1).

Table 1: Phytochemical screening of the aqueous extract of the various plants

	Mangifera indica	*Carica papaya*	*Anogeissus leoicarpus*
Resin	+	+	++
Alkaloids	+	+++	+
Saponins	+	+	+++
Tannins	+	-	+
Glycosides	-	+	++
Flavonoids	++	++	++

KEY:

- \- = absent
- \+ = slightly present
- ++ = present in moderate quantity
- +++ = present in high quantity

Toxicological Studies

Table 2 shows that all the animals gained weight during the course of the experiment.

Table 2: Effects of the Plants' Extracts At Different Doses on Rats' Weights and the Rats' Survival Rate

PLANT	DOSE (mg/kg)	B/WT (g)	WT GAIN (g)	SURVIVAL (%)
PAWPAW	10	156.87 ± 6.4	24.87 ± 8.2	100
	100	108.23 ± 14.8	21.20 ± 5.6	100
	1000	161.70 ± 26.7	20.67 ± 4.6	100
MANGO	10	130.90 ± 9.2	17.53 ± 3.4	100
	100	131.50 ± 5.2	18.40 ± 3.2	100
	1000	151.40 ± 16.9	22.37 ± 6.8	100
MARKE	10	142.30 ± 21.0	18.77 ± 4.2	100
	100	96.53 ± 3.3	21.37 ± 4.7	100
	1000	135.83 ± 28.8	18.90 ± 2.7	100
CONTROL		151.27 ± 19.3	17.60 ± 1.9	100

Table 3 shows the effect of oral administration of the plants' extracts on the biochemical parameters that are markers of kidney function. The data obtained are compared to the control at $P < 0.05$. There is no significant difference between the control and the test groups.

Table 3: Kidney Function of Rats Fed With Plants' Extract (Acute Toxicity 1st Stage)

Parameter		Plant Extract			
	Control	Pawpaw	Mango	Marke	
	□±SEM	X̄±SEM	X̄±SEM	X̄±SEM	
Sodium (mmol/L)	4.33±0.03	4.42±0.40	4.38±0.04	4.27±0.04	
Potassium (mmol/L)	104.00±1.15	105.78±0.60	103.33±0.58	103.11±0.82	
Chloride (mmol/L)	23.00±1.00	22.33±0.83	21.89±0.51	23.11±0.45	
Bicarbonate (mmol/L)	5.37±0.23	5.80±0.23	5.39±0.14	11.32±5.84	
Creatinine (mmol/L)	81.33±1.76	79.22±1.43	83.22±1.89	79.33±1.76	
Total Protein (g/dl)	71.00±0.58	72.11±1.11	70.39±0.63	73.67±1.04	

$P < 0.05$ is considered significant

Table 4: Liver Function of Rats Fed With Plants' Extract (Acute Toxicity 1st Stage)

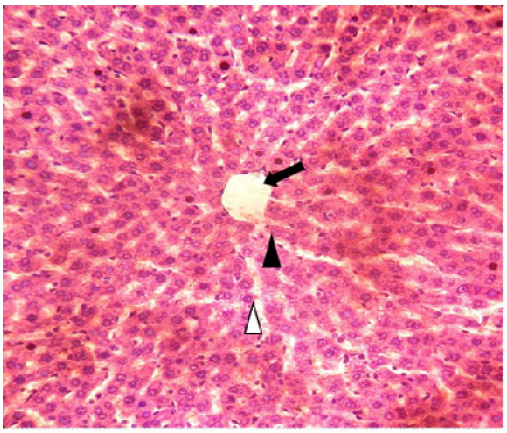
Parameter		Plant Extract			
	Control	Pawpaw	Mango	Marke	
	□±SEM	□±SEM	□±SEM	□±SEM	
ALB	36.00±2.08	38.22±0.68	33.83±1.01	38.56±2.10	
TB	10.06±0.07	10.00±0.00	10.00±0.00	10.00±0.00	
CB	5.03±0.03	5.00±0.00	5.00±0.00	5.00±0.00	
ALK	82.00±1.15	87.67±1.48	83.56±1.21	84.22±1.00	
AST	15.17±0.23	12.10±0.55	13.83±0.43	15.16±0.22	
ALT	11.17±0.62	11.05±0.93	10.60±0.70	11.56±0.56	

$P < 0.05$ is consider significant

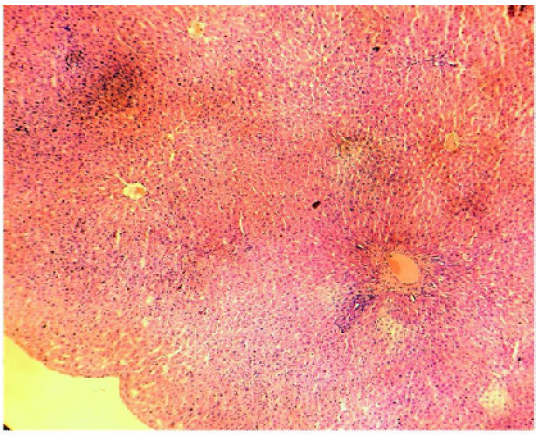
Table 5: Haematological Analysis of Rats Fed With Plants' Extract (Acute Toxicity 1st Stage)

Parameter		Plant Extract			
	Control	Pawpaw	Mango	Marke	
	□±SEM	□±SEM	□±SEM	□±SEM	
HB(g/dl)	17.40±1.00	17.58±0.38	16.71±0.53	17.17±0.44	
PCV(%) (HCT)	43.73±4.62	49.24±1.73	46.83±1.40	45.16±1.00	
RBC($\times 10^{12}$/L)	8.49±0.27	8.56±0.24	8.17±0.23	8.09±0.17	
MCHC(g/dl)	356.00±15.87	358.14±9.40	351.56±7.80	373.00±10.68	
WBC($\times 10^{9}$/L)	8.15±0.37	7.80±0.25	8.19±0.26	8.44±0.16	
Platelet ($\times 10^{9}$/L)	434.00±107.70	449.11±47.05	406.90±58.92	408.06±57.98	
Granulocyte (%)	87.90±0.55	88.36±0.43	88.21±0.32	87.79±0.25	
Lymphocyte (%)	9.40±0.49	9.09±0.42	9.16±0.29	9.41±0.25	
Monocyte (%)	2.70±0.12	2.56±0.08	2.62±0.04	2.72±0.04	

$P < 0.05$ is consider significant



Micrograph of Liver tissue section showing normal appearing hepatic lobule with a centrilobular vein (*Black Arrow*) from which radiates chords of hepatocytes, in-between which are the hepatic sinusoids (*White arrowhead*) . Occassional Kupfer cells (*Black arrowhead*) are seen. *Haematoxylin and Eosin stain (x40)*

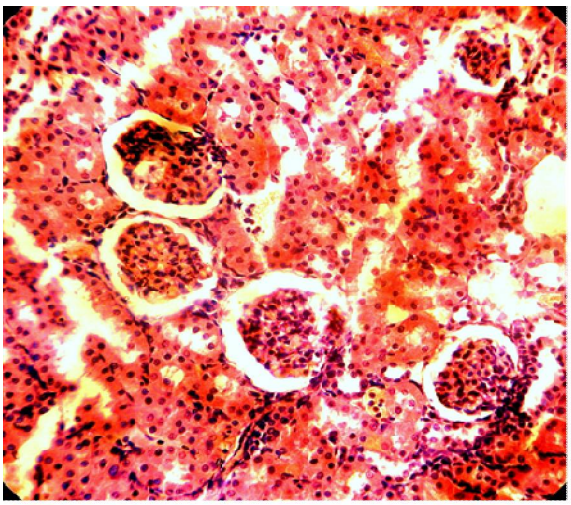


Low power magnification of Liver tissue section showing a preserved hepatic lobular architecture. Periportal Inflammatory infiltrates can be observed at the lower right portion. *Haematoxylin and Eosin (X 10)*

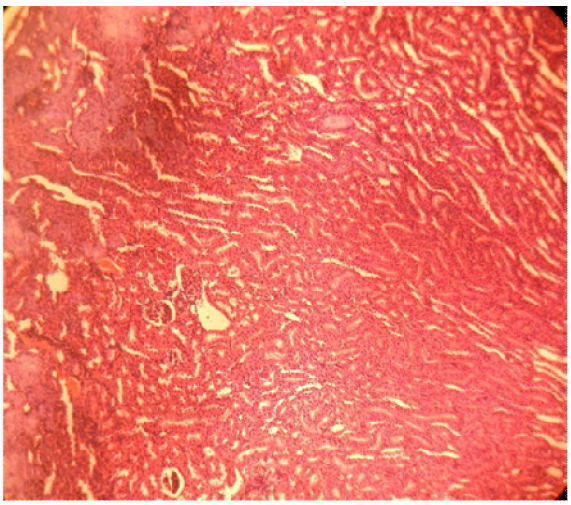
Plate 1: Micrograph of Liver Tissue

Plate 1 shows the photomicrographs of liver tissue section of one of the control animals at ×40 and ×10 magnification. The tissue appear normal showing the characteristic centriolubular vein (see the black arrow) from which radiates chords of hepatocytes. The liver tissue section from the test animals compare well with that of the control, even in the groups fed with a dose of the plants' extract of 5000mg/kg of the rat's body weight.

Plate 2 shows the ×40 and ×10 magnifications of the photomicrograph of a rat's kidney tissue section from the control group. The renal section is normal appearing and compare well with that of the test animal groups.



Photomicrgraph of Control Kidney Section showing normal appearing renal tissue. *H and E (Original Magnification X40)*



Photomicrgraph of Control Kidney Section showing normal appearing renal tissue. *H and E (Original Magnification X10)*

Plate 2: Micrograph of Kidney Tissue

Discussion

Herbal medicine is an integral form of therapy and is in the bedrock of medication in Nigerian society. It is therefore of great importance to study these plants with a view of supplying information on their LD_{50}. The three plants in this study are boiled together in water overnight and given to the patients to drink and bathe with to rid themselves of typhoid fever. This study is born out of concern of the deleterious effect of consumption of these herbs

without taking dosage into consideration. Toxicological studies are carried out in different experimental animals to forecast the toxicity and safety of drugs and herbal products for human and animal consumption. The LD_{50}, which is the index of acute toxicity, is not considered a biological constant because studies from different laboratories have revealed variations in LD_{50} values **[9]**. Factors such as sex, age, genus, strain, diet and environment temperature, can affect LD_{50} values **[10]**. Although medicinal plants may produce

various biological activities in humans, few have been studied regarding their toxicity.

In this study, the results of the acute toxicity testing revealed no mortality in all of the groups (Table 2), indicating that the aqueous extract of *anogeissus leiocarpus* leaf, *carica papaya* leaf and the stem bark of *mangifera indica* exert protective effects when taken orally. The works of Agaie *et al* (2007), Nwiloh *et al* (2007) and Ogbe *et al* (2012) corroborated with the finding in this study. Table 2 also shows that there was a mean body weight increase both in the control groups and in the groups fed with the plants' extracts this could mean that the animals were in good physical state. No toxic symptoms was observed during the two weeks duration of the experiment indicating that these herbs are non toxic and safe for the rats even at 5000mg/kg body weight. This assumption is supported by biochemical, haematological and histological findings which revealed a non significant statistical changes in values obtained in the test and control groups.

Table 3 shows the effect of the crude plants' extracts on the rats' kidneys. Kidney functions were evaluated by means of serum urea and creatinine levels (Wingard et al., 2000 ABDALLAH ETA L).There were no significant differences between the control and the test groups. Though the value of creatinine in the group that received pawpaw leaf extract was higher than that of the control groups, it was not statistically different. Table 4 shows the effect of the extracts on the rats' liver. Two enzymes mainly associated with hepatocellular damage in liver are aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Witthawaskul et al. 2003). However, AST is present in a wide variety of tissues including heart, skeletal muscles, kidney, brain and liver (Mukinda and Syce, 2007). Elevation in plasma AST activity accompany damage to liver and other tissues (13) while elevation in plasma ALT activity is seen mainly in liver damage. ALT is present in enclosed compartments of the liver and they are particularly useful in measuring hepatic necrosis, especially in small animals (Cornelius, 1989). The non-elevated levels of AST and ALT in this study may be an indication of no hepatic damage caused by the administration of the crude plants' extracts in this research work. The animals given pawpaw crude extract had a non significant higher value of alkaline phosphatase than the control group. Alkaline phosphatase (ALK) is a marker for liver cholestasis (16…1361901719).

Table 5 shows the effect of these plants' extract on the rats' haematological parameters. The hematopoietic system is an important target for toxic substances **[11]** and is a significant indicator of the physiological and pathological condition in animal and man **[12]**. In this study, these plants' extracts had good effects on the hematopoietic and leucopoietic system as seen in the values obtained. The packed cells volume (PCV) of the groups fed with the plants' extract are non significantly higher than the control groups.

These crude extracts of the three plants appear to have good effects on the rats. This could be because of the rich content of secondary metabolites found these plants (Table 1). The presence of phytochemical constituents in medicinal plant parts have been reported (Trease and Evans, 1989;Sofowora, 1993; Evans, 1997). The histopathological findings (Plates 1 and 2) supported the results obtained from haematology and biochemical parameters on the safety of oral consumption of the aqueous extract of *anogeissus leiocarpus* leaf, *carica papaya* leaf and the stem bark of *mangifera indica* even at a high dose of 5000mg/kg.

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11/9/2014