African Journal of Pharmacy and Pharmacology Research Vol. 1(2) pp. 017-022, April 2011
Available online http://www.interesjournals.org/IRJPP

Full Length Research Paper

Effect of crude extracts of *blighia unijugata* on histology of the liver and kidney of adult wistar rats

[1]Aquaisua N. Aquaisua, [2]Rosemary B. Bassey*, [3]Bassey M. Ikpeme, [4]Enobong I. Bassey

[1]Department of Anatomy, Faculty of Basic Medical Sciences, University of Uyo – Nigeria,
[2]Department of Anatomy, Faculty of Basic Medical Sciences, University of Uyo – Nigeria*,
[3]Department of Community Medicine, University of Calabar Teaching Hospital, Calabar – Nigeria, [4]Department of Anatomy, Faculty of Basic Medical Sciences, University of Uyo – Nigeria.

22 March 2011.

Reports have been made that the ingestion of unripe *Blighia sapida* (ackee) fruits may be responsible for poisoning resulting in a substantial number of unexplained deaths. This study was carried out to investigate the effect of *Blighia unijugata,* a sister specie of *Blighia sapida* on the histology of the liver and kidney. Twenty male rats were divided randomly into four groups of five rats each. Group 1, the control received daily oral dose of distilled water. Group 2, 3 and 4 were administered 424 mg/kg, 849 mg/kg and 1273 mg/kg of *Blighia Unijugata* respectively for 4 weeks. Phytochemical screening of *Blighia Unijugata* was done. Weekly body weight of the rats was taken throughout the duration of this experiment. The organs were processed for histology. The preliminary phytochemical screening of *Blighia unijugata* extract showed the presence of tannins, saponins, flavonoids, cardiac glycosides and anthraquinones. There was a statistically significant increase in the body weight of animals in control group and in the animals dosed with 849 mg/kg BU. No histopathological changes were observed in the liver and kidney when compared to control. Therefore, *Blighia unijugata* is not toxic to the liver and kidney of Wistar rats.

KEY WORDS: *Blighia unijugata*, Toxicity, Liver, kidney

INTRODUCTION

Despite many achievements in human health care in the twenty-first century, many of the world's population in developing countries lack regular access to affordable essential drugs. For these people, modern medicine is never likely to be a realistic treatment option; in contrast, traditional medicine is widely available and affordable, even in remote areas (Zhang, 2000; Tabuti, 2004).

A source of human poisoning is through the use of self-medicated herbal remedies which may contain plant toxins. This type of poisoning is more common in cultures that believe in traditional indigenous medicines like Africa; but cases have also occurred in more developed countries where interest in use of herbal medicine is growing as people endeavour to use natural remedies (Eddleston *et al*., 2003).

Traditional medicine blends readily into the socio-cultural lifestyles where it is deeply rooted. Thus, it remains popular in many developing countries due to its inherited and strong belief systems (Sofowora, 1982; WHO, 2002). In 1964, the Organisation for African Unity developed the Scientific and Technical Research Commission (OAU/STRC) and organized the Inter-African Symposium (in 1968 in Dakar) on the

Development of African medicinal plants. The symposium decided that the efficacy of herbs used by traditional health practitioners should be tested (AACHRD, 2002).

Blighia unijugata Bak is a tree indigenous to the forests of West Tropical Africa. It is usually small but sometimes attains 35 meters in height. It belongs to the *Sapindaceae* family and has different species: *Blighia sapida* (Ackee), *Blighia unijugata* and *Blighia welwitschii*.

It is planted for shade and is attractive in appearance, having red or pinkish-yellow fruit. The wood is used for

*Corresponding authoe email: rosemary_bassey@yahoo.com
Tel: 234-8064853304

buildings; bark pulp as an enema or is macerated by draught and taken as febrifuge and purgative. The fruit has common use as a fish poison and the seed infusion is given in case of sickness and vomiting. The seeds, because of their oil content, and the jacket because of its potash content, are burned and the ashes used in making soap. It is also recognized for its sedative and analgesic properties in treatment of rheumatism (Burkill, 2000).In Jamaica, the fruit serves as a major component of the national dish, ackee (*Blighia sapida)* and codfish. There, the fruit is also processed in brine, canned and exported earning over US $13 million annually.

In recent years, there have been reported incidences of toxic hypoglycaemic syndrome in West Africa. Lethality was 100% in the Burkina Faso epidemic and victims of the illness were all children (Barennes *et al*., 2004). Deaths were linked to *Blighia sapida* (ackee) intoxication due to enhanced concentrations of di-carboxylic acids in the urine of the victims. It has been suggested that the ingestion of unripe *Blighia sapida* (ackee) fruits may be responsible for a substantial number of unexplained deaths in pre-school children in West Africa (Meda *et al*., 1999).

Therefore, the study was carried out to determine the effect of *Blighia unijugata* on the histology of the liver and kidney of Wistar rats.

MATERIALS AND METHODS

Collection and identification

The ripe fruits of *Blighia unijugata* were purchased from the local market in Uyo. It was authenticated by a taxonomist in the Department of Botany of the University of Uyo.

Preparation of seed extract

The seeds were dried in an oven (temperature of between 30 – 40^{o}C) for a week. The dried seeds weighed 1.15 kg and Soxhlet extraction was carried out using 1 Liter of methanol as solvent at the Pharmacognosy Department of Faculty of Pharmacy, University of Uyo. At the end of the extraction process, the extract was further dried in an oven regulated at 38^{o}C and the yield which was 105.23 g.

Animals

Twenty adult male Sprague-Dawley rats, 16-20 weeks old and weighing between 105-200 g were used for this experiment. The rats were procured from the Animal House of the Faculty of Basic Medical Sciences, University of Uyo and authenticated by a taxonomist at the Zoology Department of the same University. They were kept in the Animal House of the Faculty of Basic Medical Sciences under standard conditions of temperature (25 ± 5°C), with a 12 hour light: 12 hour dark cycle to acclimatize for one week before the commencement of the experiment. They were allowed unrestricted access to water and commercial rat pellets (Pfizer Feeds PLC, Ikeja).

Experimental protocol

The twenty male rats were divided randomly into four groups of five rats each. The experimental groups received daily oral doses of the drugs as follows: Group 1, the control received daily oral dose of distilled water by oral gavage. Group 2, 3 and 4 were administered 424, 849 mg/kg and 1273 mg/kg of *Blighia Unijugata* respectively for 4 weeks. Weekly body weight of the rats was taken throughout the duration of this experiment. All procedures involving animals were performed in accordance with the guidelines guiding the use and care of laboratory animals and approved by the Departmental Committee on the use and care of animals.

Sample collection

Animals were anaesthetised using chloroform at the end of the experiment.

Histological procedures

The organs were processed for histological work as follows: the liver and kidney from each animal was fixed in 10% formol saline. The fixed tissues were transferred to a graded series of ethanol and then cleared in xylene. Once cleared, the tissues were infiltrated in molten paraffin wax in the oven at 58^{o}C. Serial sections of 5 micrometres thick were obtained from a solid block of tissue, cleared, fixed in clean slides, stained with Haematoxylin and Eosin stains and examined with the light microscope.

Phytochemical screening

Phytochemical screening was done by a modification of method described by Sofowora (1984), Trease and Evans (1989), Harbone (1973) and Wall *et al* (1952). Crushed sample weighing 230 g were extracted with 1.0 L of solvent, using the Soxhlet extractor for at least 6 hours until the complete extraction has occurred. The obtained extract was further evaporated to dryness using the vacuum rotary evaporator. The extract was phytochemically screened for the following active constituents: saponins, tannins, flavonoids, anthraquinones and cardiac glucosides**.**

Determination of LD_{50}

A preliminary LD_{50} of *Blighia unijugata* seed extract was determined using the fixed–dose procedure described

Table 1: Phytochemical screening of *Blighia unijugata*

Test	Observation	Inference
Saponins	Froth	++
Tannins	Blue-Black Precipitate	+++
Flavonoids	Orange Colour And Effervescence	+++
Cardiac Glycosides	Reddish-Brown Interface	+++
Anthraquinones	-	+
Lierberman's Test	Green Ring	++

Table 2: Effect of *Blighia unijugata* on weekly body weight (g)

Groups	Week 1	Week 2	Week 3	Week 4
1	135.0±26.8 [a]	134.9±27.5 [a]	140.1±29.3 [a,b]	142.6±29.8 [a,b]
2	142.9±38.5 [a]	143.3±39.6 [a]	145.1±40.1 [a]	148.1±45.2 [a]
3	131.3±19.4 [a]	132.9±18.9[a]	136.9±18.6 [a,b]	142.4±19.1 [a,b]
4	132.4±24.5 [a]	133.6±27.1 [a]	136.4±27.2 [a]	137.4±26.8 [a]

The results are Mean ± Standard deviation, n=5

by Walum Erik in 1998. The *Blighia unijugata* seed extract was given at one of three fixed doses at a time to 5 males Sprague-Dawley rats (S-D).

Statistical analysis

Results were expressed as mean ± standard deviation. Analysis was carried out using one-way analysis of variance (ANOVA) and the Scheffe's post hoc test on Microsoft Excel 2007. The level of significance was considered at $p < 0.05$.

RESULTS

The preliminary phytochemical screening of *Blighia unijugata* extract showed the presence of bioactive components like tannins, saponins, flavonoids, cardiac glycosides and anthraquinones (Table 1).

LD_{50} of *Blighia unijugata* seed extract determined was 4242.64mg/kg body weight of rat. At the dose of 4242.64mg/kg, there were clear sign of toxicity with mortality of 50% of the rats.

Table 2 shows the effect of *Blighia unijugata* on the weekly body weight of the control and experimental groups. There was a statistically significant increase in the body weight of animals in the control group (group 1) from 135.0±26.8 to 140.1±29.3 in week 3 and 142.6±29.8 in week 4. There was no statistically significant difference in the weekly body weight of the animals in group 2. The animals in group 3 also showed a statistically significant increase in weight from 131.3±19.4 in week 1 to 136.9±18.6 in week 3 and 142.4±19.1 in week 4. In group 4, there was no statistically significant difference in the body weight of the animals

Effect of *Blighia unijugata* on histology of the liver and kidney

No clinical signs of toxicity were observed in any of the groups during the experimental procedures. Histological changes were checked in the liver and kidney of all the groups. There were no significant changes observed in liver and kidney when compared to control (figures 1-8).

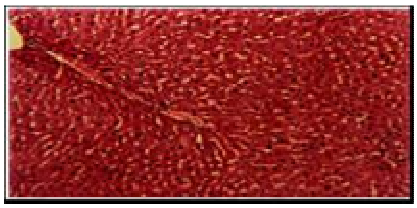
DISCUSSION

In the present era, plant and herb resources are abundant, but these resources are dwindling fast due to the onward march of civilization (Vogel, 1991). Although a significant number of studies have been used to obtain purified plant chemical, very few screening programmes have been initiated on crude plant materials. It has also been widely observed and accepted that the medicinal value of plants lies in the bioactive phyto-components present in the plants (Veeramuthu *et al*., 2008).

There have been diverse reports on the poisoning resulting from consumption of *Blaghia sapida* (ackee). Seidlein (1913) reported 62 cases of vomiting sickness in Jamaica with venous congestion and hyperaemia observed in various organs of people dying from *Blaghia sapida* poisoning. Scott (1916) administered arils of unopened ackee to kittens which resulted in the death of these animals. Microscopic examination of the stomach, ileum, liver and kidney showed hyperaemia.

In this study, *Blighia unijugata*, another species from the *Sapindaceae* family, after microscopic examination however showed no toxicity to the liver and kidney when administered in different doses to Wistar rats for a period of four weeks. Only one death was recorded

(i)



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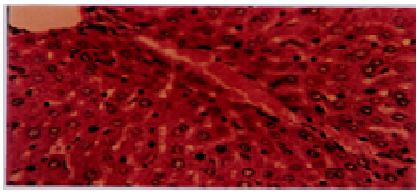
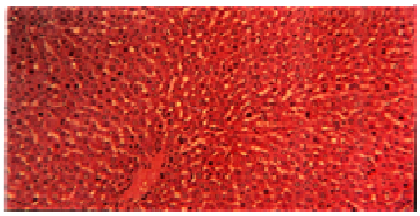


Figure 1: Section through the liver of Group 1 (control) at magnification (i) x 100 and (ii) x400 stained with Heamatoxylen and Eosin

(i)



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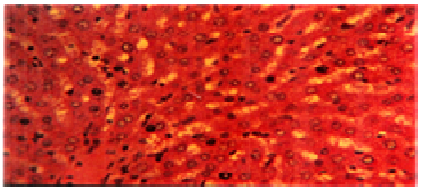
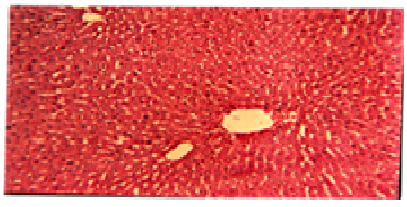


Figure 2: Section through the liver of Group 2 at magnification (i) x 100 and (ii) x400 stained with Heamatoxylen and Eosin

(i)



(ii)

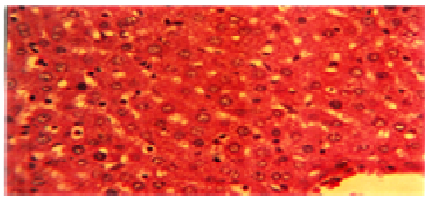
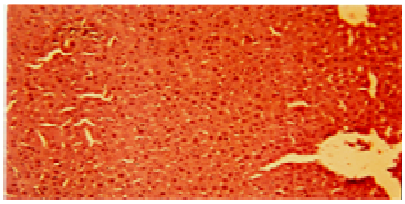


Figure 3: Section through the liver of Group 3 at magnification (i) x 100 and (ii) x400 stained with Heamatoxylen and Eosin

(i)



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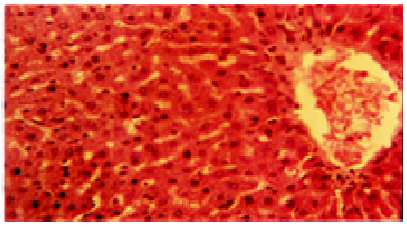
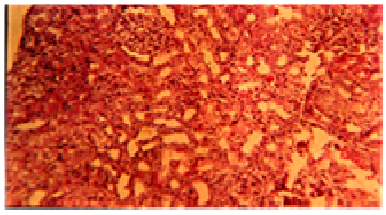


Figure 4: Section through the liver of Group 4 at magnification (i) x 100 and (ii) x400 stained with Heamatoxylen and Eosin

(i)



(ii)

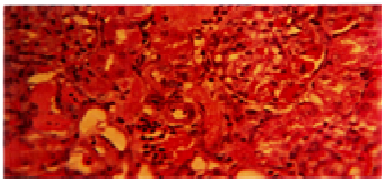
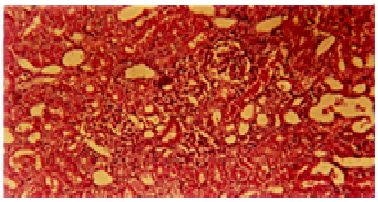


Figure 5: Section through the kidney of Group 1 (control) at magnification (i) x 100 and (ii) x400 stained with Heamatoxylen and Eosin

(i)



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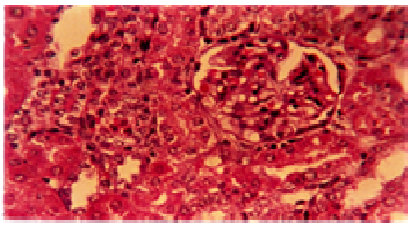
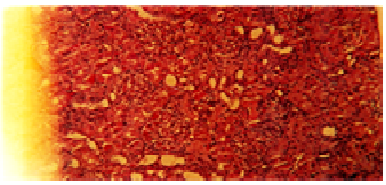


Figure 6: Section through the kidney of Group 2 at magnification (i) x 100 and (ii) x400 stained with Heamatoxylen and Eosin

(i)



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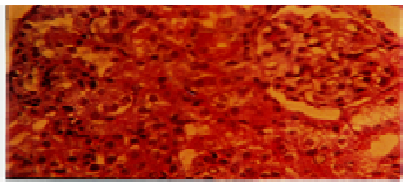
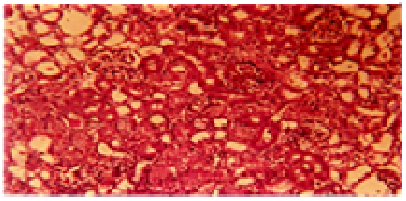


Figure 7: Section through the kidney of Group 3 at magnification (i) x 100 and (ii) x400 stained with Heamatoxylen and Eosin

(i)



(ii)

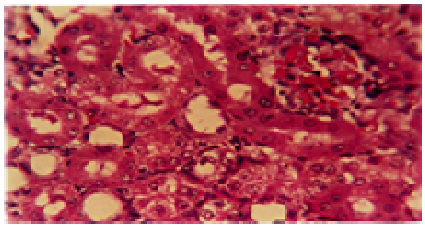


Figure 8: Section through the kidney of Group 4 at magnification (i) x 100 and (ii) x400 stained with Heamatoxylen and Eosin

which was due to the extract getting into the air passage of the animal.

Jordan and Burrows (1937) believe that the ackee poison is a glucoside and that it is present in both the seeds and pods of both ripe and unripe ackees. According to these investigators, the ackee contains, in the early stages of development of the fruit, a saponin which is haemolytic; and that phytosterol later fixes the saponin and renders it non-toxic.

Most saponins, which readily dissolve in water, are poisonous to fish. Therefore in ethno-botany, saponins are primarily known for their use by indigenous people in obtaining aquatic food sources, of which *Blighia unijugata* and *Blighia sapida* is commonly used for in the Southern part of Nigeria. Saponin-glycosides are very toxic to cold-blooded organisms, but apparently not to mammals (Hostettmann and Marston, 1995; Hall and Walker, 1991).

CONCLUSION

This is a purely experimental animal study. From the data obtained, *Blighia unijugata* is not toxic to the liver and kidney of Wistar rats. Further studies into *Blighia unijugata* are however needed.

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