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# TESTICULAR AND RENAL TOXICITY IN RATS ADMINISTERED EXTRACTS OF *HEINSIA CRINITA* LEAVES

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## ABSTRACT

Crude aqueous extracts of the black variety of *Heinsia crinita* leaves, a vegetable commonly consumed in southern Nigeria, were orally administered to albino rats of Wistar strain for seven days. Organopathology of test animals showed no histological changes in liver, heart and brain tissues, compared with control. However, kidneys and testes of test animals showed significant changes in cytological ultra-structure. The paper discusses the possibility of renotoxicity as well as reproductive toxicity to males of human consumers, by extrapolation.

## INTRODUCTION

The tropical forests of Nigeria abound with lush vegetation, many of which are consumed by indigenes. Many have not been botanically developed, nor have their chemical compositions, anti-nutrient profiles and medicinal values been researched into (Isong and Essien, 1996). These are fundamental scientific issues, needful in order to provide consumers with information on the nutritional and anti-nutritional consequences of their vegetable diets, as well as provide a database for agriculturists, pharmaceutical personnel and dieticians (Isong and Idiong, 1997).

*Heinsia crinita* (Rubiaceae; Ibibio: atama), is one of such vegetables and is widely consumed by the peoples of southern Nigeria. Recently, the dried, packaged leaves have become export items to overseas countries as a gourmet delicatessen vegetable. Indeed the plant is reported to be as far spread as the tropical belts of East, South and West Africa, with both the tree bark and roots, possessing some medicinal properties (Dalziel, 1936; Watt and Breyer-Brandwijk, 1962; Roda, 1986). Locally, the native traditional herbalists in southeastern Nigeria allege that branch stalks are used in oral hygiene as well as in the prevention and cure of decaying gums; roots are used for the cure of gonorrhoea and other STDs, as well as serve as abortifacients; tender leaves are used as anti-pruritics, while mature leaf extracts are used to cure many stomach disorders, including peptic ulcer. Oral administration of the concentrated leaf liquor are lethal, (U.J. Ekott, pers. Comm.). Unpublished observations by Etuk *et al* in 1997 in which Wistar albino rats were fed with leaf diets of the black and white *Heinsia crinita* varieties, showed that test animals died after three days of feeding the black but not white variety. Overt toxicity signs observed prior to the death included hair loss and enervation. The young men who smoke the dried leaves allege that 'it makes them put up a brave behaviour in the face of challenge'. In certain of the ethnic groups in the region, the leaves are regarded as food taboo for men, but not women because of its alleged dampening effect on libido, amongst other undesirable effects (U.J.Ekott; A. Williams, pers comm.).

Several local varieties of this shrub exist (Etuk et al 1998 ) and leaves of all such varieties consumed, are processed by cutting and washing, as well as the addition of a local, crude culinary bicarbonate, prior to culinary preparations for consumption. The addition of large quantities of palm oil and ground sea-foods like crayfish, to soups prepared from the leaves, also probably detract from any possible

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toxic side-effects. Consumers in the rural areas allege that there exist about six or more locally edible varieties of this green leafy vegetable but literature regarding the botanical establishment of such varieties and their nutritional and medicinal profiles is scarce (Etuk et al 1998). Recently some work has begun on this tropical shrub, regarding the issues presented (Etuk *et al*, 1998). This paper reports the organopathology to experimental rats, following the oral administration of aqueous extracts of the so-called edible black variety of the *Heinsia crinita* vegetable. The report is preliminary and more research is presently on-going to establish certain crucial clinical, biochemical and physiological parameters, using both male and female animals.

## MATERIALS AND METHODS

***Preparation of extracts from dry leaves.***

Fresh young leaves of the edible black variety of *Heinsia crinita* were harvested from the forest in Nung Udoe, Ibesikpo Local Government Area of Akwa Ibom State of Nigeria. They were washed, air-dried and subsequently oven-dried (Gallenkamp; 50°C/72hrs.). Dry leaves were manually micro-particulated in a porcelain mortar to a mesh size of 150mm. The sample was extracted using a Soxhlet quickfit EX 5/55 extractor, with methanol: ethanol solvents (v/v, 70:30). A heating mantle was used to evaporate the extract to dryness. Thereafter, a fifty-percent solution was made using distilled water and administered to experimental rats.

***Preparation of extract from fresh leaves.***

Fresh, washed and air-dried leaves were weighed and mashed using 100mls distilled water in small portions. The fresh aqueous extract was subsequently squeezed through cheesecloth and the 100ml extract orally administered to experimental rats. Even though the extract of *Heinsia crinita* leaves have been shown to possess wide anti-microbial activity (Ekpa and Ebana, 1991), care was taken to prepare, preserve and administer the extract in hygienic and sterile conditions, in order to preclude microbial contamination.

***Animal experimentation.***

Albino rats of Wistar strain were obtained from the University animal house. Rats were six weeks old and weighed between 38 to 80 grams. The animals were bred and maintained on rat pellets (Pfizer Nigeria Ltd.) with water provided *ad libitum*and with a day and might cycle of 12h, average room temperature of 28 ± 0.1°c. Fifteen male rats were divided into three groups. The first group of five (control group) was dosed orally daily, with 0.2ml of distilled water for seven days. The second group of five were dosed orally daily, with 0.2mls of the fifty per cent aqueous *Heinsia crinita* leaf extract for seven days, while the third group of five were orally dosed with 0.2ml of the undiluted aqueous extract for seven days, also. Animals were sacrificed by numbing with chloroform fumes and organs excised, washed in normal saline, weighed and fixed in Bouin's fluid/100% formaldehyde/6hrs.

***Histology.***

Tissues were selectively fixed, dehydrated, cleared and embedded in paraffin wax. Mounting, sectioning and placing of tissues on slides, followed. Slides were appropriately stained and cleared, while examination of slides followed, using a Kyona microscope 753548.

## RESULTS

Figs. 1-6 are photomicrographs of various tissues from the animals.

***Testis:*** Significant changes are observed. Testes of control animals show normal histology (Fig. 1). Seminiferous tubules are lined by stratified germinal epithelium and spermatogenic cells are severally layered with different stages of spermatozoid development. The lumen of seminiferous tubules is filled with mature sperm and interstitial tissue shows numerous ledgic cells.

Group two animals administered 50% aqueous extract (Fig.2), show adversely altered testes. The germinal epithelium is not differentiated into layers. Rather, clumps of cells appear with densely stained nuclei, indicating cytotoxicity of an apoptotic nature. Interstitial tissues are also poorly developed and there is a striking absence of ledgic cells.

Group three animals administered 100% of the fresh crude extract (Fig. 3), showed adverse changes more drastic than those of group two

Fig. 1: Control Testis (x 110)

Fig. 2: Testis of 50% Crude Extract (x 110)

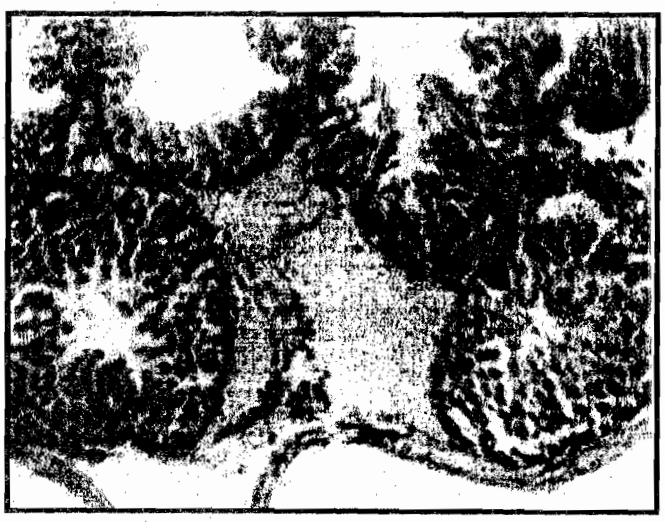

Fig. 3: Testis of 100% Crude Extract (x 110)

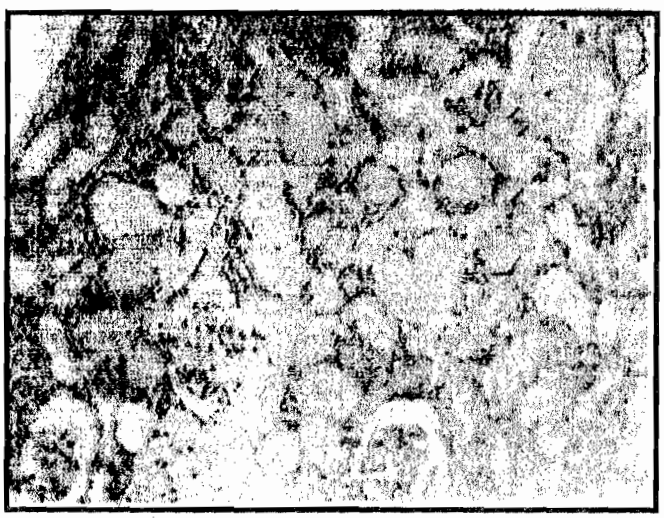

Fig. 4: Control Kidney (x 110)

Fig. 5: Kidney of 50% Crude Extract (x 110)

Fig. 5: Kidney of 100% Crude Extract (x 110)

testes. There is gross distortion of germinal epithelium and seminiferous tubules contain scanty and immature spermatozoa. Interstitial tissues are distorted and occur in patches, while ledgic cells are also absent.

***Kidney:*** Renal tissues of test animals showed significant adverse alteration compared with control. Glomeruli of control tissues show normal capsular structure (Fig. 4). There is however a gradual reduction in capsular space, of the Bowman's capsule from the 50% to the 100% extract-affected tissues (Fig. 5 and 6). The Bowman's capsule shows additional inflammation.

***Brain:*** There is no significant change in brain tissue of test animals, compared with control. The cerebral cortex slides all appear normal.

***Liver:*** There is no significant change in tissues of test animals, compared with control. Hepatocytes show normal histology from the central vein.

***Heart:*** No significant changes were observed in cardiac tissues of the animals.

## DISCUSSION

Observations made in this study were rather dramatic in that after only a brief exposure of seven days, substantial testicular and renal damage in experimental rats, occurred. This probably indicates that an extremely toxic chemical is present in the plant, as alleged by traditional lôcal herbalists. It is speculated that the observed renotoxicity in rats orally administered the aqueous extract, may have resulted from the fact that the kidney, being a detoxificatory organ, may play a key role in the concentration of the active toxicants and is thus itself susceptible to toxicity. The liver is the major organ for xenobiotic metabolism in the body. However, many xenobiotics are processed primarily by the kidneys (Baco, 1971; Kalant and Rochalau, 1989). It is remarkable therefore that no ultra-structural changes were noted in the livers of rats, while substantial damage occurred in kidneys. It must be noted however, that the *Heinsia crinita* leaves are usually processed prior to human consumption, by cutting, washing and cooking together with certain condiments such as palm oil and ground (cray)fish, which may provide some ameliorative protection from toxicity in humans. None of these food-processing procedures were effected during the animal feeding trials of the present study. *Heinsia crinita* leaves are a delicacy widely consumed among the people of southeastern Nigeria. Indeed, so well loved is this vegetable, that it has recently been dried, ground and packaged for export overseas. Yet no such mention of similar toxic observations has been reported in literature Subsequent oral interviews conducted extensively by the authors among some elderly and knowledgeable rural dwellers as well as traditional herbalists, however revealed that the vegetable is taboo for males in certain clans of the *Annag* ethnic group of Akwa Ibom State, as well as the Ikom of Cross River State, Nigeria because it undermines both penile erection and sexual performance in men.

Statistics (UMC, 1998) show that 90% of cases of barrenness reported at the University of Uyo Medical Centre are secondary in nature, arising from male, rather than female sterility. A recent study conducted among 5800 infertile couples, further identified men as the major cause of infertility in over half of the couples studied (The Guardian, 1998). It is unfortunate that even till today, women are blamed or even divorced because of their so-called 'barrenness' in this society. The possibility of a dietary male contraceptive advertent or otherwise, arises from the results of this study; where women could apply the unprocessed leaves as 'a secret weapon against reproductive demands at home'. However, research work regarding contraceptives, male or female, are often repressed and do not receive the support of pharmaceutical companies; they are therefore not encouraged (Bilger, 1998).

Research work is in progress to investigate the toxicants of the observed testicular and renal toxicity, and its possible mechanism of action, with a view to clinical studies in the long term. Variation in the period and dose of administration will also be tested.

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## REFERENCES

Baco, Z. M., 1971. Fundamentals of biochemical pharmacology. Pergamon Press, N.Y. pp 498 - 503.

Bilger, B., 1998. The secret garden. *The Sciences* 38(1):38-43.

Dalziel, J. M., 1936. The useful plants of West Africa. Crown Agents, London. pp. 15 - 20

Ekpa, O. and Ebana, R., 1991. Chemical composition and antimicrobial properties of leaves of four Nigerian plants with flavouring principles. *Trop. J. Appid. Sci* 1:30-34.

Etuk, E.U.I., Bassey, M.N., Umoh, U.O., Inyang, E.G., 1998. Comparative nutritional studies on three varieties of *Heinsia crinita. Plant varieties and seeds.* 11:151-158

Isong, E. U. and Essien, I. ,1996. Nutritional and anti-nutritional composition of three varieties of Piper species. *Plant Food for Human Nutrition* 49: 133-137.

Isong, E. U. and Idiong, U. I., 1997. Comparative studies on the nutritional and toxic composition of three varieties if *Lesianthera africana. Plant foods for Human Nutrition.* 51(2):79-84.

Kalant, H. and Roechalau, W.H.E 1989. Principles of Medical Pharmacology. 5th edn. B.C. Decker Inc. Toronto. pp. 9-15.

Röda, M.A.P., 1986. Some Medicinal plants of African and Latin American Forests.

Resource Development Branch Food and Agric. Organization (FAO) United Nations Italy. pp 5 -23.

The Guardian 1998. Study identifies men as major cause of infertility. *The Guardian Newspapers.* 14 (7149): 5.

UMC 1998. University of Uyo Medical Centre Records, University of Uyo, Uyo - AKS, Nigeria.

Watt, J.M. and Breyer-Brandwijk, M.G., 1962. The medicinal and poisonous plants of South and Eastern Africa. E. S. Livingstone, Edinburgh. pp. 1-7.