



PHYTOCHEMICAL SCREENING AND HISTOPATHOLOGICAL STUDIES ON THE SEEDS OF *COLOCYNTHIS CITRULLUS* IN ALBINO RATS

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ABSTRACT

The present study was designed to carry out phytochemical screening and histopathological studies. Four groups of animals (A, B, C and D), each containing 3 albino rats (Wistar strain). Group A, B and C were fed with different concentrations of amended diet (25%w/w, 50%w/w and 75%w/w) of dried powdered seeds of *Colocynthis citrullus* respectively. The last group (D) served as a control and was fed with a standard diet. The animals were housed in separate cages, kept in a clean environment and provided with food and water *ad-libitum*. Prior to the commencement of the experiment, the animals were pre-conditioned for two weeks. On the 91st day of the experiment, the animals were sacrificed and subjected to post-mortem examination. The organs were removed, processed and microscopically examined. The various plant extracts (Pet-ether, methanol and ethanol) were subjected to various phytochemical tests to identify the constituents present. The preliminary Phytochemical screening of the various plant extracts revealed that *C. citrullus* seeds contain alkaloids, steroid glycosides and flavonoids. Gross examination showed no observable lesions in any of the tissues and organs of the rats in both the treated and control groups. Microscopically, the testicles of the rats in all the groups were observed to be actively involved in spermatogenesis. In the kidneys, renal casts and calcified mass were seen. Also periglomerular and intertubular mononuclear cellular infiltrations were found in some of the kidneys, whereas in the intestine, necrosis of the intestinal villi, some involving the glands while some were not noticeable. This work highlights the phytochemical constituents and the histopathological changes brought about by chronic consumption of the dried powdered seeds of the plant in rats.

Keywords: Histopathology, phytochemical screening, *Colocynthis citrullus*, albino rats

INTRODUCTION

Colocynthis citrullus Thunb. is a slender hairy monoecious annual, often sprawling over the ground. It is from the family Cucurbitaceae. The plant is commonly known as bitter melon or bitter melon and is locally called 'agushi' in northern Nigeria. It is mostly cultivated in middle belt areas of Nigeria like in Niger state, Kaduna state, etc. It is marketed either as broken pieces, which are light and spongy, or in the form of flakey

powder; which is yellowish-orange or yellowish-green in colour Dalziel (1963). Ethnomedicinally, the seed yields about 12.7-17% by weight of fixed oil, capable of being used as an illuminant and is said to be some time used for blackening of gray hair (Watt and Breyer-Brandwijk, 1962). The oil is used in India as a remedy of snakebite, scorpion sting and epilepsy and to stimulate the growth of hair. The reported phytochemical

constituents of *C. citrullus* seed are given as, phytosterol, phytosteroline and alpha spinasterol (Watt and Breyer-Brandwijk, 1962).

The seed of *C. citrullus* is also reported to have diuretic activity (Vohora and Khan, 1981). The chromatographic fraction given to rats at a concentration of 0.1mg/ml showed insulin release stimulation on pancreas using glucose induced insulin secretion (Nmila *et al*, 2000). When 10% of seed was mixed in chicks' ration, the following changes were noticed in the serum; alkaline phosphatase stimulation, creatin kinase stimulation and lipid synthesis stimulation. Also hepatotoxic activity was found on the liver of the chicks (Bakhiet and Adam, 1995). The methanolic extract; from the seed at a concentration of 100µg/kg had protease (Hepatitis C Virus) inhibition and antibacterial activity. Susceptible organisms include; *Bacillus substillis*, *Escherichia coli* and *Aspergillus terreus*. It has weak antiyeast activity (Hussein *et al*, 2000; Bashir *et al*, 1992). The seed of *Colocynthis citrullus* is consumed in large quantities because of its medicinal and nutritional values in Nigeria. In view of the common use of this plant, both as food and as herbal medicine, this paper reports the phytochemical constituents and pathological effects of *Colocynthis citrullus*.

MATERIALS AND METHODS

Plant material

Fresh seeds of *Colocynthis citrullus* were collected during the rainy season (September, 2004) in Zaria, Kaduna State, Nigeria. The plant was identified at the Herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria where a voucher specimen (No.1266) was deposited. The seeds were air-dried, powdered and stored in an airtight container prior to use.

Phytochemical Screening

One hundred grams of the air dried powdered plant material was weighed and continuously extracted with petroleum ether in a soxhlet extractor and subsequently with methanol. Three grams each of the various extracts, were subjected to preliminary phytochemical screening using standard methods specified by (Brain and Turner, 1975; Trease and Evans, 2004).

Experimental Animals

Albino rats comprising of mixed sexes were used. The animals were housed in separate cages, kept in a clean environment and provided with food and water *ad-libitum*. Prior to the commencement of the experiment the animals were pre-conditioned for two weeks. The animal care and handling was conducted in compliance with the National Regulations for Animal Research. Ahmadu Bello University's Ethical Committee reviewed the protocols, and found it to be consistent with International Animal Welfare Guidelines.

Treatment of animals

Twelve albino rats of mixed sexes weighing between 110 - 220g were randomly allocated into four groups (A, B, C and D) of three animals each. The animals were allowed free access to water and food pellets. The rats were kept at room temperature in 12hr-12hr light and dark cycles. Animals in groups A, B and C were fed with the 25%, 50% and 75% (w/w) formulated diets respectively. The animals in the control (group D) were fed with the standard diet (Feed Master[®]) grower mash. The experiment lasted for a period of 13 weeks.

Preparation of Organs Samples

On the 92nd day of the experiment, the animals were sacrificed; their various tissues were removed, processed and were examined grossly and microscopically following the method of (Arthur and John, 1978).

RESULTS

Phytochemical Screening

Three Phytochemical constituents were

identified in *Colocynthis citrullus* seeds as shown in table 1.

Table 1: Phytochemical constituents of the various extracts of *Colocynthis citrullus* seed

Constituents	<i>Colocynthis citrullus</i>
Alkaloid	+
Tannins	-
Reducing Compounds	-
Anthracenosides	-
Coumarin Derivatives	-
Saponins	-
Steroid Glycosides	+
Flavonoids	+
Polyuronides	-
Carotenoids	-

Keys: + = Present; - = Absent

Histopathological Studies

Gross Lesions

Various tissues and organs of both the treated and the control groups were carefully observed and showed no observable gross lesions.

Microscopic lesions

The rats that were treated with the amended diet that contained 25% *Colocynthis citrullus* had necrosis of the intestinal villi involving the intestinal glands. Mononuclear cells infiltrated the necrotized areas (plate I). The liver of one rat in the group had focal areas of necrosis of the hepatic cells (plate IV). However, the livers of the other rats in the group had no observable microscopic lesions. This may be as described as idiosyncratic effect since no effect was observed in the other 2 group members. The testicles of the rats in the 25% group were actively involved in spermatogenesis (plate II). The pancreas of rats had focal areas of necrosis of the pancreatic cells (plate III). There were no observable microscopic lesions in the brain,

lung, heart, spleen and stomach, of the rats in the 25% group.

The rats that were treated with the amended diet that contained 50% *Colocynthis citrullus* had necrosis of the intestinal villi involving the intestinal glands. Mononuclear cells infiltrated the necrotised areas (plate VI). The kidneys of the rats in the group had focal areas of glomerular and tubular necrosis with periglomerular mononuclear cellular infiltration (plate VII). There were renal casts and some calcified mass collecting tubules. A single roundworm in the microscopic field of rat number 4 was observed. The livers of the rats had focal areas of necrosis of the hepatic cells and haemosiderosis (plate VIII). The testicles of the rats in the group were actively involved in spermatogenesis (plate V). There were no observable microscopic lesions in the brain, spleen, pancreas, stomach, heart, and uteri of the rats in the group.

The rats that were treated with the amended diet that contained 75% *Colocynthis citrullus* had necrosis of the intestinal villi involving the intestinal glands. Mononuclear cells

infiltrated the necrotised areas (plate IX). The kidneys of all the rats in the group had glomerular and renal tubular necrosis, periglomerular and intertubular mononuclear cellular infiltration (plate X), renal casts and calcified mass in the collecting tubules (plate XI). There were focal areas of necrosis of pancreatic cells (plate XII). The livers of the rats had fatty degeneration and necrosis of the

hepatic cells. The testicles of the rats in the group were actively involved in spermatogenesis. There were no observable microscopic lesions in the lungs, hearts, brains, spleens and stomachs.

There were no observable microscopic lesions in the brains, lungs, testicles, intestines, stomachs, pancreas hearts, spleens and livers in the control group.

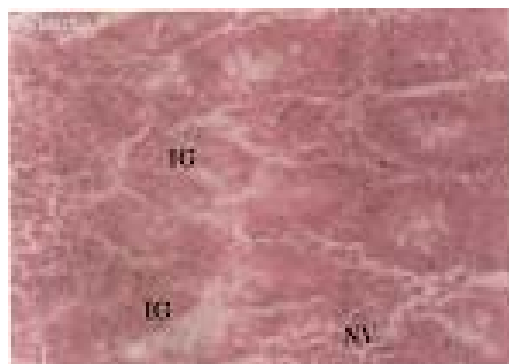


Plate I: Photomicrograph of a section of intestine from rat treated with amended diet containing 25% *C. Citrullus* powdered seeds, H & E stain, (X 400). Note the necrotized intestinal villi (NV) involving the intestinal glands (IG)



Plate II: Photomicrograph of a section of testis from rat treated with amended diet containing 25% *C. Citrullus* powdered seeds, H & E stain, (X 400). Note the active spermatogenesis (ST) by the spermatogenic cells

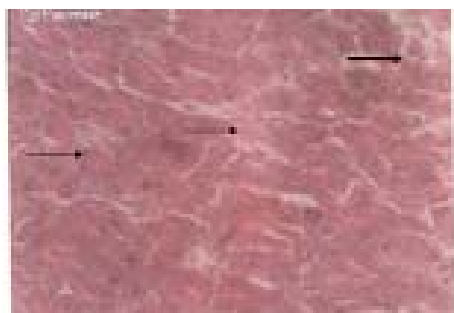


Plate IV: Photomicrograph of a section of liver from rat treated with amended diet containing 25% *C. Citrullus* powdered seeds, H & E stain, (X 400). Note the focal area of necrosis of the hepatic cells (FN)



Plate III: Photomicrograph of a section of pancreas from rat treated with amended diet containing 25% *C. Citrullus* powdered seeds, H & E stain, (X 400). Note the focal area of necrosis of the pancreatic cells (arrows)



Plate V: Photomicrograph of a section of testis from rat treated with amended diet containing 50% *C. Citrullus* powdered seeds, H & E stain, (X 400). Note the active spermatogenesis by the spermatogenic cells in the seminiferous tubule (ST)

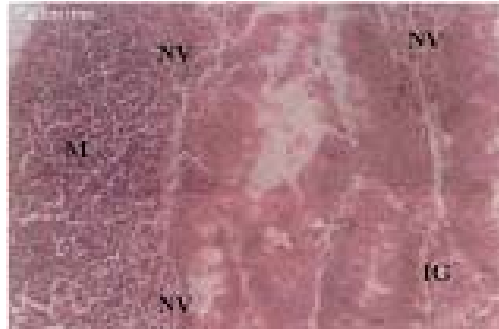


Plate VI: Photomicrograph of a section of intestine from rat treated with amended diet containing 50% *C. Citrullus* powdered seeds, H & E stain, (X 400). Note the necrotized intestinal villi (NV) involving the intestinal glands (IG) infiltrated by mononuclear cells (M)

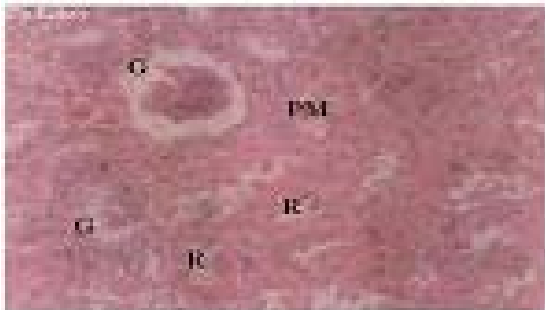


Plate VII: Photomicrograph of a kidney from rat treated with amended diet containing 50% *C. Citrullus* powdered seeds, H & E stain, (X 400). Note the glomerular (G) and renal tubular (R) necrosis and periglomerular mononuclear cellular infiltration (PM)



Plate VIII: Photomicrograph of a section of liver from rat treated with amended diet containing 50% *C. Citrullus* powdered seeds, H & E stain, (X 400). Note the focal area of necrosis of the hepatic cells (FN)

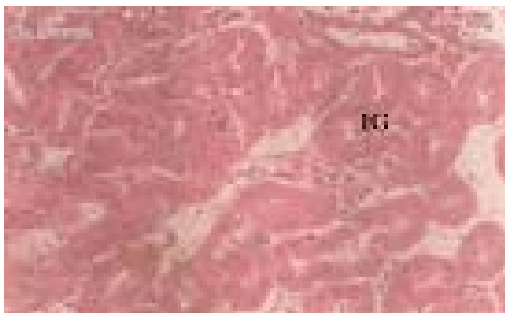


Plate IX: Photomicrograph of a section of intestine from rat treated with amended diet containing 75% *C. Citrullus* powdered seeds, H & E stain, (X 400). Note the necrotized intestinal villi involving the intestinal glands (IG)

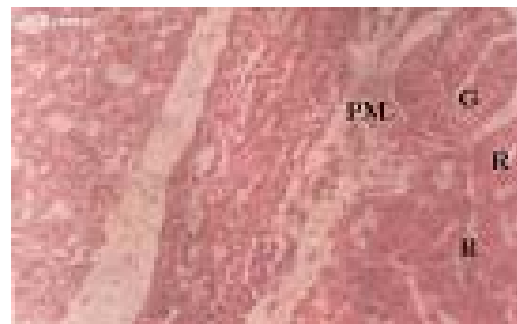


Plate X: Photomicrograph of a kidney from rat treated with amended diet containing 75% *C. Citrullus* powdered seeds, H & E stain, (X 400). Note the glomerular (G) and renal tubular (R) necrosis and periglomerular mononuclear cellular infiltration (PM)

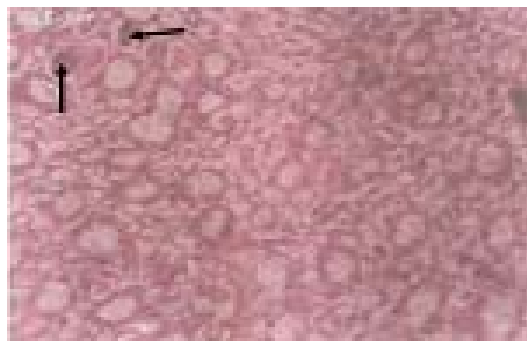


Plate XI: Photomicrograph of a kidney from rat treated with amended diet containing 75% *C. Citrullus* powdered seeds, H & E stain, (X 400). Note the renal casts in the renal medullar (arrow heads)



Plate XII: Photomicrograph of a section of pancreas from rat treated with amended diet containing 75% *C. Citrullus* powdered seeds, H & E stain, (X 400). Note the focal areas of necrosis of the pancreatic cells (FN)

DISCUSSION

Phytochemical screening is usually carried out to screen for and to characterized the constituents available in a given plant sample. Generally, in the phytochemical screening of any plant one normally identifies secondary metabolites that have accumulated to some extent at specific organ of the plant. These metabolites that are mainly used by the plant for protection against herbivores may have pharmacological activity when tested on animals. Result of phytochemical screening of the seed of *Colocynthis citrullus* of the various extracts showed the presence of alkaloids, steroidal glycosides and flavonoids.

Histopatological study of medicinal plants is an aspect of Pharmacognosy in Nigeria that has not been fully developed. In orther words is an area in Nigeria that has not been given much attention in order to determine the toxic or beneficial effect(s) of the medicinal plants when used for a long time as food or as medicine, to various tissues and organs of the body. The result of the work showed one beneficial and two harmful effects that are consistent with the treated group of animals

fed with *Colocynthis citrullus* seeds. The beneficial effects were observed in the testes of the animals showing or rather carrying out active spermatogenesis, which is quite different from what was observed in the control group. The harmful effects were seen in the kidneys and intestines but the stomach of all the animals appeared normal.

It could therefore be concluded that indiscriminate large consumption of seed of *Colocynthis citrullus* collected from around Zaria Local Government Area, Kaduna State, Nigeria to be consumed as both food and medicine is not safe for a long period of time.

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