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Phytochemical Screening and Histopathatology of Aqueous and Ethanol Leaf Extracts of Bitter Gourd (*Momordica Charantia*) on Experimental Albino Rats Organs

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Abstract:
The effect of aqueous and ethanol leaf extracts was determine against histology of liver, kidney and spleen. Qualitative phytochemical analysis of aqueous and ethanolic *M.Charantia* leaf extracts was carried out. Albino rats were grouped in a five group of five rats each and distributed randomly. The first group was given 2ml of normal saline, remain groups were treated with 200mg/kg body weight and 400mg/kg body weight of aqueous and ethanolic leaf extracts *M.Charantia* for 21 days. After the experimental period, the rats were sacrifice and organs (liver, kidney and spleen) were collected for histopthalogical analysis. Qualitative phytochemical analysis shows the presence of alkaloids, flavonoids, saponins, glycosides, steroids, tannins, terpenoids and phenols. Histopathological analysis of spleen shows hyperlassia of the red pulp in groups treated with 200mg/kg body weight of aqueous leaf extract and white pulp hyperplasia in group treated with ethanolic extracts when compared to normal controls and is dose-dependent. Group treated with 200mg/kg body weight of aqueous extract shows mild glomerular degeneration compared to normal group. Enlarge vascular channel and congested in group treated with 400mg/kg body weight aqueous extract in liver while group treated with 400mg/kg body weight of ethanolic extract shows enlarge partal trait vascular congestion with inflammatory cells within congested blood fibrous tissue when compared to normal group liver. In conclusion, aqueous and ethanolic *Momordica Charantia* leaf extracts has mild effect on the organs of the rats.

Keywords: Phytochemical Screening; Histopathology; Aqueous and Ethanol Leaf Extracts; *Momordica Charantia*; Phytochemical analysis.

Introduction

Momordica charantia is a species of *Momordica* belonging to the cucurbitaceae family with the common name, bitter mole, bitter gourd or bitter squash, fruit (English). In Adamawa state it grows as a weed and in most tropical countries. It is not formally cultivated as a commercial crop anywhere in the world, but has been a seem-domesticated volunteer crop in home gardens or on fertile land (Makgakga; 2004). It is normally cooked with pounded groundnut (peanut butter) and beans to serve as dish and to improve the flavour.

Medicinal plants contain some organic chemicals which produce definite physiological action (s) on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids, and flavonoids (Edeoga *et al.*, 2005). All over the world several hundreds of plants have been identified as good source of medicinal agents and are used in traditional medicine for several selected therapeutic purposes, such as antibacterial and antifungal agent. Traditionally, the usage of plant in curing illnesses has deep root in human history (Edeoga *et al.*, 2005).

The medicinal plants of Africa accounts for nearly two third of the total plants species used in modern system of medicine and in rural areas as tea, extracts. Herbal drugs are widely prescribed, even when their biological ingredients are not known, due to their effectiveness, fewer side effects and low cost (Kumar *et al.*, 2009; Ajayi *et al.*,2011). The rational design of novel drugs from traditional medicine obtained from plant offers new prospects in modern health care (Manjamalai *et al.*, 2010). The aim of this study is to determine the effect of M. Charantia on histopathology of kidney, liver and spleen.

Materials and Methods

Preparation of Aqueous and Ethanol Extract of M. Charantia

Sample collection and Authentication

Fully matured dark green leaves of *M. Charantia* were collected in and around the vicinity of Mubi North Local Government, Adamawa State. The plant species was authenticated in the State Ministry of forestry, Mubi Adamawa State.

Extraction of crude extract

The plant leaves was thoroughly washed with tap water and rinse with distilled water to remove dust and other unwanted materials accumulated on the leaves from their natural environment. The dust free leaves were shade dried and pounded/pulverised using pestle and mortar to powdered form.

The powdered leaves (100g) were weighed and soaked in 350mL of ethanol and water in a volumetric flask. The flasks with instantaneous shaken were corked and left to stand for 48hrs at room temperature. After 48hrs, the mixtures were filtered using Watman Filter Paper No. 1, the filtrate were concentrated using Rotary evaporator and water bath at 38^{o}C to dryness (Evans, 1996).

Phytochemical Screening

The leaves extract were subjected to preliminary phytochemical screening using methods described by (Evans and Trease 1996); (Sofowara and Harbone 1990). Various qualitative chemical tests were conducted for detection of alkaloids, phytosterols, terpenoids, phenols, steroids, flavonoids, tannins, carbohydrates, glycosides and saponins in aqueous and ethanol extract of the leaves.

Alkaloid detection Test

Solvent free extract was mixed with few mL of dilute acid and boiled few minutes, filtered and the filtrate was tested with various alkaloid reagents:

Test for Tannins

Ferric chloride test: 1mL of plant extract was diluted with distilled water and 2 drop of 5% ferric chloride was added. A transient greenish to black colour indicates the presence of tannins.

Wagner's reagent: 1.27g I and 2g KI was dissolved in 5mL of water and made up to 100mL with distilled water. Test: 1ml of plant extract one to two drop of Wagner's reagent was added by the side of the test tube, a white-creamy or yellow coloured precipitate indicates a positive result.

Test for glycosides

Extracts were hydrolised with dil. HCl and then subjected to test for glycosides. Modified Borntrager's Test: extract were treated 5% ferric chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in ammonical layer indicates the presence of glycosides.

Test for Steroids

To 1mL of plant extract, 1mL chloroform and 3 drop of conc. H_2SO_4 were added to form a layer. A reddish-brown interface shows the presence of steroids.

Phenols detection test

Ferric chloride test: 1mL of plant extract was added to 2mL of distilled water followed by 5 drops of 10% ferric chloride. Formation of bluish black colour indicates the presence of phenols.

Test for flavonoids

a. Lead acetate test: 2mL of plant extract was treated with 4 drop of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.
b. Aluminium chloride test: 4mL of the filtrate was shaken with 1mL of 1% aluminium chloride solution. Formation of yellow coloration precipitate was observed which indicates the presence of flavonoids.

Test for Saponins

Form test: 2mL of the extract was diluted with 2mL distilled water. The mixture was shaken vigorously. Formation of foam persists for 10 minutes which indicate the presence of saponins.

Result

The result of the phytochemical screening of aqueous and ethanol extract of *M. Charantia* provided on Table 1 reveals the presence of alkaloids, carbohydrate, flavonoids, glycoside, saponin, , steroids, tannins, terpenoids, and phenol, while Table 2 shows the results of Histopathological changes in the kidney, liver and spleen after oral administration at dose of 200mg/kg bwt. and 400mg/kg bwt. aqueous and ethanol leaves extracts.

Table 1: Phytochemical analysis of *M. Charantia* of aqueous and ethanol leaves extracts

Chemical Constituents	**Aqueous**	**Ethanol**
Alkaloids	+	+
Flavonoids	+	+
Glycoside	+	+
Saponin,	+	+
Steroids,	+	+
Tannins	+	+
Terpenoids,	+	+
Phenol.	+	+

+ Sign shows detection level of the phytochemicals present in extracts

Table 2: Effect of *Momordica Charantia* extracts on histology of rat's kidney, liver and spleen after oral administration at dose of 200mg/kg bwt. and 400mg/kg bwt. Aqueous and ethanol

				Histo-pathological changes observed		
S/No.	group	extracts	Dose oral	Kidney	Liver	spleen
1.	Control	Distilled water	2ml	NAD	NAD	NAD
2.	Test	Aqueous	200mg/kg bwt.	AD	AD	AD
3.	Test	Aqueous	400mg/kg bwt.	AD	AD	AD
4.	Test	Ethanol	200mg/kg bwt.	AD	AD	AD
5..	Test	Ethanol	400mg/kg bwt.	AD	AD	AD

NAD = No abnormality detected

AD = Abnormality detected

The effects of *M. Charantia* extracts on histology of rat's spleen, kidney and liver after oral administration at dose of 200mg/kg bwt. ALE, 400mg/kg bwt. ALE is shown in Figs.1, 2 and 3, respectively.

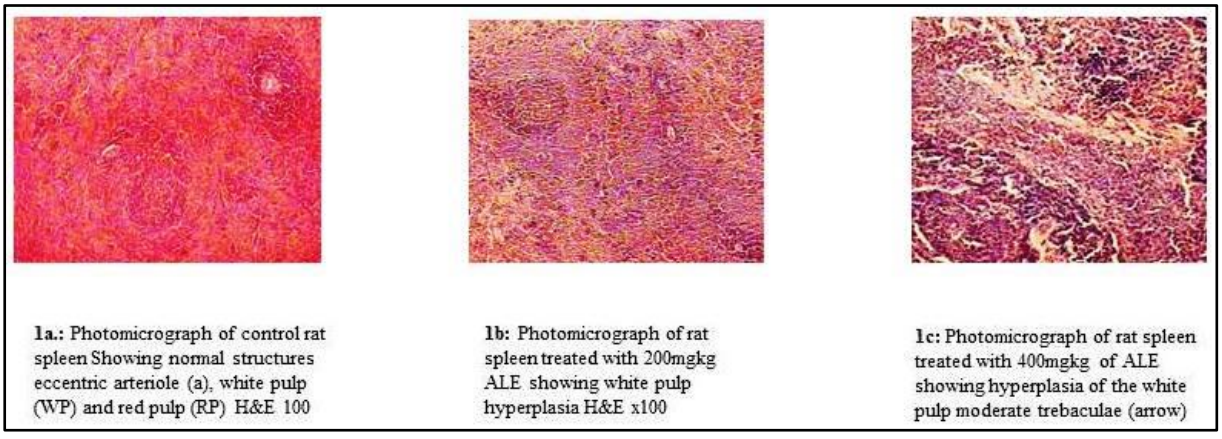
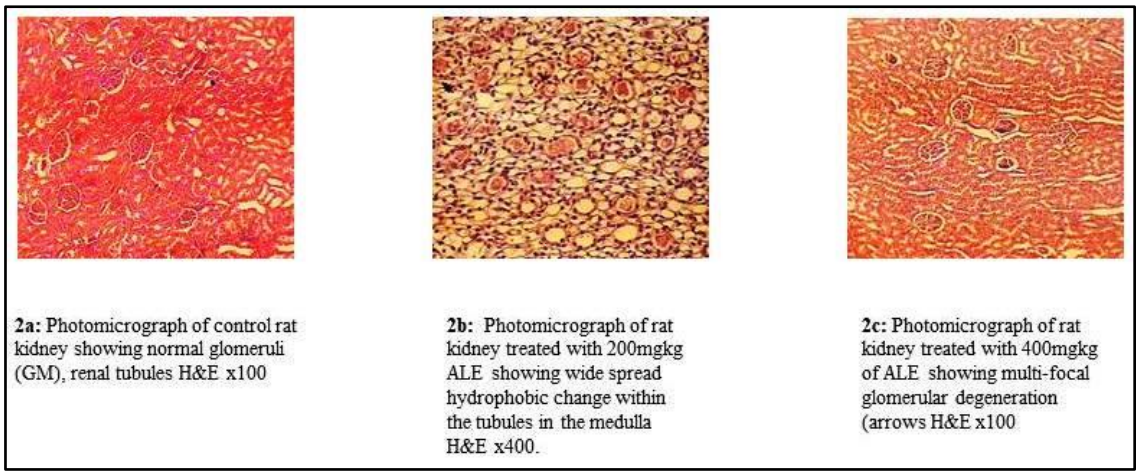


Figure 1: Effect of *M. Charantia* extracts on histology of rat's spleen after oral administration.

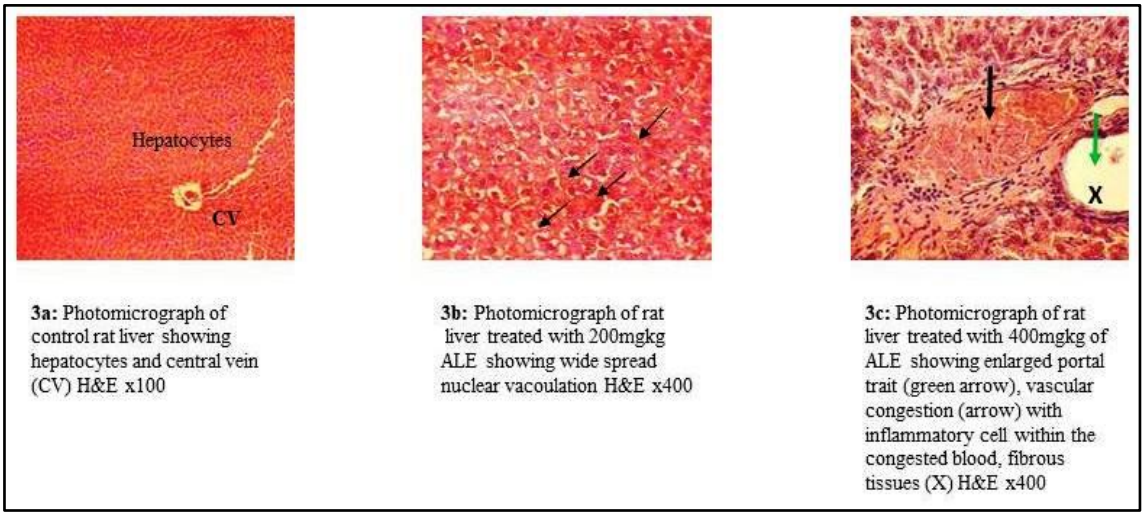


2a: Photomicrograph of control rat kidney showing normal glomeruli (GM), renal tubules H&E x100

2b: Photomicrograph of rat kidney treated with 200mgkg ALE showing wide spread hydrophobic change within the tubules in the medulla H&E x400.

2c: Photomicrograph of rat kidney treated with 400mgkg of ALE showing multi-focal glomerular degeneration (arrows H&E x100

Figure 2: Effect of *M. Charantia* extracts on histology of rat's Kidney after oral administration.



3a: Photomicrograph of control rat liver showing hepatocytes and central vein (CV) H&E x100

3b: Photomicrograph of rat liver treated with 200mgkg ALE showing wide spread nuclear vacoulation H&E x400

3c: Photomicrograph of rat liver treated with 400mgkg of ALE showing enlarged portal trait (green arrow), vascular congestion (arrow) with inflammatory cell within the congested blood, fibrous tissues (X) H&E x400

Figure 3: Effect of *M. Charantia* extracts on histology of rat's Liver after oral administration.

Discussion

Preliminary studies on quantitative phytochemical analysis of aqueous and ethanolic leaf extracts of *Momordica Charantia* revealed the presence of Alkaloid, Carbohydrate, Flavonoid, Glycoside, Saponins, Steroid, Tannins, Tapenoids and Phenols. Phytochemicals are plant secondary metabolite that have been reported to have many biological and therapeutic uses, so *Momordica Charantia* is expected to have many medicinal uses as shown in table 4.1 (Vishma *et al.,* 2013 and Narender *et al.,* 2012).

Alkaloids are the most significant compounds play a metabolic role in the living systems and are involved in the protective function in animals. Steroids are medicinally evolved. Flavonoids have been used against the cancer causing tumors and it inhibits the promotion of growth and progression of tumors (Stevens *et al.,* 1992). Phenols when mixed with the flavonoid compounds in plants are reported to show multiple activities like antioxidant, anti-carcinogenic, anti-inflammatory e.t.c (Asha *et al.,* 2011). Tannins inhibit the pathogenic fungi and antimicrobial activity of extracts showed better activity by the presence of tannins. Saponins cause the leakage of proteins and degradation of cell wall enzymes from the cell (Zablotowicz *et al.,* 1996).

The histopathological studies of liver, kidney and spleen of both control and experimental rats were performed after oral administration of the extracts for 21 days (Table 2). No detectable differences in the histopathology of these organs of control and the extracts treated rats were observed when viewed under oil immersion objective. This

indicates that the tested extracts have effect on cells of these organs due to the highly content of saponin (Table1).

Conclusion

The aqueous and ethanol extracts of *M. Charantia* leaves are still useful in traditional herbal medicine, containing a number of phytochemicals and providing a scientific data base for further primary health care benefit.

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