PHYTOCHEMICAL CHARACTERIZATION AND BIOCHEMICAL STUDIES OF *Cissus multistriata EXTRACT* ADMINISTERED TO *Rattus novergicus*

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ABSTRACT

The leaves of cissus multistriata were collected, air-dried for two weeks and pulverized into powder; this was followed by extraction with either chloroform or water. The phytochemical screening of the extracts revealed the presence of carbohydrates, proteins, vitamin C, saponins, steroids, cardiac glycosides, lipids and vitamin E; whereas balsam, anthraquinone, tannins, alkaloids, cardenolides and phlobactannin were completely absent. The aqueous extract was administered to the experimental rats at doses of 100, 200, 800, 1600, 3200, and 6400 mg/kg body weight for three weeks. The control group were injected 5 ml physiological saline 3 times daily for 21 days. The test animals showed appreciable body weight increase when compared with the control group. The body weight increase was dose dependent. Analysis of the blood samples for enzymes activities, (indicators for the possible damage to the liver and kidney) showed that the leaf extract was slightly toxic to these organs. Measurement of enzymes activities revealed that lactate dehydrogenase, alkaline phosphatase, acid phosphatase, alanine and aspartate amino transferases activities were observed to have increased throughout with increase in dosage of the extract down the group. A part from the enzymes, other renal and hepatic profiles were monitored which included serum urea, creatinine, albumin and bilirubin. There was increase in the renal and hepatic profiles monitored and the increase was dose dependent. The result of this investigation indicated that prolonged use and at a high dosage of the extract could be deleterious to the liver and kidney.

Keywords: *Cissus multistriata, Vitaceae,* liver, kidney, albino rats, enzymes, analytes

INTRODUCTION

The truth about natural products with medicinal efficacy must not be allowed to die (Anslem, 2002). Selection of such natural products for use in orthodox medicine was not based on prior knowledge of its constituents but on factors like seasonal, availability, astronomical, mystical, religious and signatures of nature that the Native Doctor accepted as influencing his life (Anslem, 2002).

Cissus is a genius plant with over 350 species of tropical and subtropical which are chiefly woody vines of the grape Family (Vitaceae). The leaves are often fleshy and somewhat succulent and often used for medicinal purposes. They contain some bioactive compounds. These compounds are found in the leaves, roots, stem and bark (Burkill, 1985). It is a slightly fleshy climber flowers; ultimate branching of inflorescence cymose, unexpanded corolla, sub-globose leaflet; three oblong-eliptic rounded at base, sharply apiculate at apex; 4 - 7 cm long, 2 - 3 cm broad, petiolules fruits, 3-4 seeded. It is found in places like Togo, and Nigeria around river basins. Its distribution extends to Sudan, East Africa, Belgian-Congo and Angola (Burkill, 1985).

It is a well known plant to the traditional medicine practioners in Nigeria. It is called *Ojekere* or *Okanigbo* by the Igalas, *Ewebiomo* in Yoruba, *Ochekihiozeowehi* by the Ebira's in Kogi State, and *Mukala* by the Ibos in Nigeria.

It is used as medicinal plant for the treatment of diverse ailments in different locations. The Ebira's use the stem prepared in form of decoction as internal cleanser for new born babies while the Yoruba's use the leaves for the treatment of infertility in women and stomach ailment in children. It is commonly used by the Ibaji's in Kogi State for the treatment of malnutrition diseases such as kwashiorkor and marasmus in children. Other uses of this plant include its use as cough remedy, fracture healing etc.

Rattus novergicus were used in this study because they have close physiology to that of humans cheap to obtain and give a more reliable response. The objective of this study was to test the toxic effect of *cissus multistriata* leaf extract on organs of the body. This plant is used by many herbal doctors for the treatment of different ailments without proper dosage. Elevated blood levels of liver and kidney enzymes are indications of organ toxicity or tissue damage, hence their measurement in this work. This research also aims to screen the plant for its bioactive components that might be responsible for the healing properties claimed by the users.

MATERIALS AND METHODS

Preparation of leaf extract: The leaves of *cissus multistriata* were collected from Ega in Idah Local Government Area, Kogi State, Nigeria during rainy season when the plant thrives very well. The leaves

were washed to remove dirts; air dried for two weeks and then pulverized using mortar and pestle. The percolation method of extraction was employed. The powdered or pulverized plant sample was soaked in a solution of chloroform and water (3:5 v/v) for 92 hours, filtered and the filtrate evaporated to give off the chloroform and water. The solid concentrate of the extract was stored in vials.

Experimental Animal Care and Management: The experimental animals used were albino rats (*Rattus novergicus)* weighing between 210 – 274 g obtained from ABU Agricultural farm, Zaria, Kaduna State, Nigeria. The animals were grouped into six test groups of four rats each labeled 1 to 6. The seventh group served as control. All rats were fed and watered *ad libitum* using 20 % CP Guinea feed.

Crude Extract Administration: 100, 200, 800, 1600, 3200 and 6400 mg/kg body weight of rat was injected three times daily for 21 days to rats in treatment groups 1, 2, 3, 4, 5 and 6 respectively. All injectibles were dissolved in 5 ml physiological saline. Rats in treatment group 7 were injected 5 ml physiological saline, 3 times daily for 21 days.

Blood Sampling and Enzyme Activity Assay: At the end of the treatment period, rats in all groups were anaesthetized, dissected and bleed via cardiac puncture. Blood samples collected per group were centrifuged at 3000 rpm. The resultant supernatant rich serum was used for enzyme activity assay thus:
Serum acid phosphatase activity was determined based on the method of Friedman and Young (1997). Alkaline phosphatase activity was determined using the method of Rosalki *et al,* (1993). The method of Tietz (1994) was followed in the determination of serum aspartate amino transferase. The determination of alanine amino transferase was carried out following the methods of Friedman and Young (1997). Lactate dehydrogenase activity was also measured according to the method of Friedman and Young (1997). Serum urea determination was carried out using the method described by Talke and Schuber (1965). The method of Teitz (1991) was followed in the determination of serum creatinine. Similarly, serum albumin determination was carried out using the methods of Tietz (1991) and Doumas *et al.* (1971). The methods of Jendrassik and Grof (1938) and Sherlock (1951) was used in the determination of serum bilirubin.

Phytochemical Screening: The plant sample was screened for the presence of bioactive components following the methods of Sofowora (1982), Trease and Evans (1993), Brian and Anthony (1989), WHO (1998), and Finar (1974)

Data Analysis: The results are expressed as mean $\pm$ S.D. Analysis of variance (ANOVA) was used to test for the differences among all the groups at $P < 0.05$. To find out where the significant difference lies among the groups, the Duncan's multiple range test was used in comparing the means (Dixon and Massey, 1957; Sokal and Rohlf, 1969).

RESULTS AND DISCUSSION

The result of the phytochemical screening showed the presence of some bioactive compounds such as carbohydrate, cardiac glycosides, flavonoids, lipids, proteins, sponnins, steroids and vitamin C while compounds like alkaloids, anthraquinones, balsam, cardenolides, phlobactannin, tannin and vitamin E were absent in the aqueous extract. The screening of the chloroform extract revealed the presence of lipids and vitamin E (Table 1).

Table 1: Phytochemical Screening of *Cissus multistriata*

S/N	Compound	Aqueous Extract	Chloroform Extract
1	**Alkaloid**	-	-
2	**Anthraquinone**	-	-
3	**Balsam**	-	-
4	**Carbohydrate**	++	-
5	**Cardenolide**	-	-
6	**Cardiacglycosides**	++	-
7	**Flavonoids**	+	-
8	**Lipids**	+	++
9	**Protein**	++	-
10	**Phlobactanin**	-	-
11	**Saponins**	++	-
12	**Steroids**	+	-
13	**Tannins**	-	-
14	**Vitamin C**	+	-
15	**Vitamin E**	-	++

***Key:* ++ =** *presence of bioactive compounds in high concentration, + = Presence of bioactive compounds in low concentration. - = Absence of bioactive compounds.*

The aqueous extract contained more of the bioactive compounds than the chloroform extract. The chloroform extract had only the fractions that are soluble in non-polar solvents. The bioactive compound contents of this plant support its uses, in providing energy; build up of worn-out tissues and regulation of internal temperature of the body. The presence of protein, vitamins and carbohydrate justifies the use of this plant in management of malnutrition. The plant also contains vitamin E and C known as tocopherol and ascorbate respectively. Both vitamins are antioxidants and as such could be useful in free radical scavenging in living system and are essential dietary constituents for humans. Vitamin C is necessary for connective tissue and promotes the healing of wounds and fracture (Barbara, 1996). This justifies the use of the plant extract for bone fracture healing.

The phytochemical screening of the aqueous extract of this plant also revealed the presence of flavonoids. The effectiveness of this plant as an anti-oedema and anti-inflamation may be due to the presence of this bioactive ingredient. Further more, is steroid which backs up the use of this plant by indigenous people of Igala land for the treatment of infertility in both males and females.

Table 2: Mean Body Weights of Rats before and After Exposure to Dosage of *Cissus multistriata Extract*

Group	Dose (mg/kg)	Before administration	One week (g)	Two weeks (g)	Three weeks (g)
1	100	210.84 ± 2.25[a]	214.74 ± 1.37[b]	216.15 ± 1.48[b]	219.98 ± 1.69[b]
2	200	225.73 ± 2.85[b]	220.16 ± 3.86[a]	230.17 ± 4.67[a]	231.96 ± 7.42[a]
3	800	231.16 ± 0.66[c]	236.30 ± 5.60[c]	237.33 ± 4.77[a]	240.17 ± 6.46[a]
4	1600	233.13 ± 0.98[d]	237.61 ± 3.12[d]	240.83 ± 5.65[a]	242.91 ± 7.18[a]
5	3200	264.11 ± 0.99[e]	265.27 ± 1.22[e]	267.07 ± 2.16[a]	270.12 ± 1.46[a]
6	6400	274.18± 4.11[f]	284.09 ± 0.05[a]	295.48 ± 1.50[a]	301.69 ± 3.33[a]
Control	—	220.03 ± 2.70[g]	220.65 ± 2.30[a]	220.95 ± 2.22[a]	221.31 ± 2.42[a]

Mean ± S. D of twenty eight replications, Figures with the same letter superscript in a vertical column are significantly different (P < 0.05).

Table 3: Mean Values of Serum Enzyme Activities in Albino Rats

	Dose in mg/kg body	Enzymes (U/L)				
Group	weight	Serum ACP	ALP	AST	ALP	LDH
1	100	10.3± 0.50[b]	22 ± 0.82[b]	94 ± 1.41[b]	199 ± 1.63[a]	256 ± 2.50[a]
2	200	17.8 ± 0.36[c]	24 ± 0.82[c]	96 ± 0.82[a]	219 ± 1.41[a]	294± 2.16[a]
3	800	20.8 ± 0.23[a]	26 ± 0.82[a]	98 ± 0.82[a]	250 ± 0.82[a]	303 ± 2.16[a]
4	1600	24.4 ± 0.36[a]	28 ± 0.82[a]	101 ±1.41[a]	251 ± 1.41[a]	306 ±2.16[a]
5	3200	30.2 ±0.24[a]	29 ± 0.82[a]	105 ± 0.82[a]	284 ± 1.63[a]	314 ± 2.16[a]
6	6400	41.0 ± 0.46[a]	32 ± 0.81[a]	105 ± 2.16[a]	383 ± 1.50[a]	325 ± 6.16[a]
Control	—	12.4 ± 0.47[a]	20 ± 0.82[a]	91 ± 0.82[a]	149 ± 1.41[a]	322 ± 1.41[a]
Pre-treated rats -		12.39 ± 0.47[e]	20 ± 0.82[d]	89 ± 0.79[f]	148.5 ± 1.39[g]	240 ± 2.51[g]

Mean ± S. D of twenty eight replications, Figures with the same letter superscript in a vertical column are significantly different (P < 0.05).; ACP = Acid phosphatase, ALT= alanine amino transferase, AST = Aspartate amino transferase, ALP = Alkaline phosphatase, LDH = Lactate dehydrogenase

Table 4: Analyte Determination

Group	Dose mg/kg	Serum urea (mg/dl)	Serum Creatinine (mg/dl)	Serum albumin (mg/dl)	Total bilirubin (mg/dl)	Conjugated bilirubin (mg/dl)
1.	100	21 ± 0.82[b]	0.9 ± 0.08[b]	1.68 ± 0.04[a]	0.25 ± 3.03[b]	0.01 ± 0.00[a]
2.	200	22 ± 0.41[c]	1.1 ± 0.08[a]	1.68 ± 0.19[a]	0.29 ± 2.58[a]	1.01 ± 0.00[a]
3.	800	25 ± 0.82[d]	1.0 ± 0.08[c]	1.70 ± 0.12[a]	0.34 ± 2.58[c]	0.02 ± 2.58[b]
4.	1600	27 ± 0.82[a]	1.1 ± 0.26[a]	1.90 ± 0.08[a]	0.46 ± 2.28[d]	0.04 ± 2.58[g]
5.	3200	30 ± 0.96[a]	1.0 ± 0.08[d]	1.80 ± 0.08[a]	0.52 ± 0.03[a]	0.09 ± 2.58[a]
6.	6400	40 ± 0.82[a]	1.2 ± 0.08[a]	1.60 ± 0.08[b]	0.58 ± 2.58[a]	0.12 ± 2.58[a]
Control	-	25 ± 0.82[a]	0.9 ± 0.08[a]	1.60 ± 0.14[a]	0.30 ± 2.58[a]	0.03 ± 2.58[a]

Mean ± S. D of twenty eight replications, Figures with the same letter superscript in a vertical column are significantly different (P < 0.05).

Steroid according to Barbara (1996) is one of the group of hormones chemically related to cholesterol, they include estrogen, androgen, progesterone, and the corticosteroids. Appreciable increase in the body weight of the animals was observed and the increase was dose dependent (Table 2). All the test groups showed appreciable increase in body weight compared to the control. This increase was statistically significant ($P < 0.05$).

The results of the serum enzyme activities are presented on table 3. The activity of acid phosphatase increased with increase in dosage of the extract. There was a significant difference ($P < 0.05$) in the increase in activity when compared with the control. This similar trend was observed for all the enzymes assayed. The effect caused by the extract on the liver could be minimal in groups 1 and 2 whose mean values when compared with the control showed no significant difference ($P > 0.05$) and more toxic to this organ of the animals in group 3 to 6 as their mean values when compared with the control showed a significant difference ($P < 0.05$). This observation correlates with that of Gupta and Verma (1990) that *cissus quardrangularis* was not toxic at lower doses but at higher ones.

Marcus and Milton (1980), observed high acid phosphatase activity in the serum to correlate with hepatobiliary disease and disease of the reticulo-endothelial system as a result of damaged liver. The extract had highest effect on animals in group 6 that was administered the highest dosage of extract and damage to the liver might have occurred in most of the treated rats. An elevated level of these enzymes, AST and ALT in acute infection, toxic hepatitis, cirrhosis of the liver and liver neoplasm has been described (Rej and Horder, 1993; Marcus and Milton, 1980). Animal in groups 5 and 6 had the highest serum enzyme activity and hence more damage must have been done to them compared with other groups and the control.

Similarly, alkaline phosphatase and lactate dehydrogenase activities were observed to have increased with increase in dosage of extract.

These enzymes activity signifies more damage to these organs and vice versa as the presence of these enzymes in the serum is an indication that these organs were affected. The result of the serum urea

concentrations showed that there was gradual increase in the serum urea concentration as the dosage increased (Table 4). There was no significant difference (P> 0.05) when group 1 was compared with the control. Comparing group 6 of dose 6400 mg/kg body weight with the control, a statistically significant difference (P < 0.05) increase was observed. An increase in serum urea in conjunction with a concomitant increase in serum creatinine levels may be an indication of kidney malfunction (Teitz, 1991).

There was increase in serum creatinine concentration with increase in dosage of extract (Table 4). Creatinine levels depend on the glomerular filtration rate (GFR). Serum creatinine is doubled when GFR is considered to be halved. The increase in serum creatinine level in this study may not have significant consequences to kidney. The result of serum bilirubin concentration as shown on table 4 reflects that increase was dose dependent. Bilirubin concentrations are effective sources of measurement of liver function. The increase in the bilirubin concentration showed that the plant extract had a degree of toxicity to the liver. This is in support of the view of Weiss *et al* (1983).

The low serum concentration of the renal and hepatic profiles monitored is an indication that the plant may be safe at lower doses and could be deleterious at higher doses. From the overall results, it may be inferred that the plant extract was slightly toxic. Despite the numerous benefits derived from this plant in terms of its medicinal values, prolonged use should be disallowed. The presence of bioactive compounds is contributory to its medicinal value. The effect of this extract on the two organs showed that the plant extract toxicity was dose dependent as indicated by the levels of enzyme activities, which were used as indicators of renal and hepatic diseases.

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