BIOCHEMICAL RESPONSE OF NORMAL ALBINO RATS TO THE ADDITION OF AQUEOUS LEAVES EXTRACT OF *HIBISCUS CANNABINUS* AND *MURRAYA KOENIGII* IN RATS DRINKING WATER

James SA[1*], MJ Ladan[2], Auta R[1], Audu J and Garba A

[1]Department of Biochemistry, Kaduna State University, Kaduna - Nigeria
[2]Department of Biochemistry, Usmanu Danfodiyo University, Sokoto -Nigeria

[*]E-mail: sjgamong@yahoo.com

ABSTRACT

Experiments were conducted to determine the biochemical effect of *Hibiscus cannabinus* and *Murraya koenigii* extracts on normal albino rats using standard methods. Analyses carried out indicated that the aqueous leaf extract of *H. cannabinus* and *M. koenigii* exhibited significant hypolipideamic activity in normal rats. Results of phytochemical studies showed that flavonoids and glycosides are the major chemical constituent of the leaf extract. Overall results indicate a significant (P<0.05) reduction of serum cholesterol, triglycerol at both concentration of 5 and 10 mg/Kg b.wt. No significant effect is seen in the hematological indices, serum glucose, Aspartate transaminase (AST) and Alanine transaminase (ALT). The significance of this study is thus discussed.

Key words: *Hibiscus cannabinus*, *Murraya koenigii*, flavonoid, Hypolipidemic

INTRODUCTION

Generally, in Nigeria like other developing countries where flora remain unexploited, plant research is repositioning drug research, hence useful drugs could be isolated from plant in this region (Fransworth *et al*; 1985). Many plant species are used in folk medicine to abate or heal various ailments, which researchers are trying to verify and implicate them to resolve the many reported metabolic disorder so as to serve as an alternative means to arrest such disorder; thereby reducing the burden of cost of treatment and possibly a better and effective drugs for such aliment.

There is the increase number in the prevalence of metabolic diseases such as diabetes mellitus due to population growth, aging, urbanization, and prevalence of obesity and physical inactivity (Wild *et al*; 2004). Associated effect of diabetes mellitus is characterized by hyperglycemia, inappropriate metabolism of carbohydrate, protein and fat. Apart of these other several factors including hyperlipidemia are involved in the development of cardiovascular complication that usually occur in diabetes resulting in morbidity and death (Sundarrajan *et al*; 2011). Plant such *Murraya koenigii* and *Hibiscus cannabinus* are widely acclaimed remedies for the treatment of diabetes (Sundarrajan *et al*; 2011; Tembhurne and Sakarkar, 2009), and are use as either vegetable or spices as the case may be in most Nigerian dishes.

Hibiscus cannabinus is a plant that is widely distributed in Nigeria and is commonly known as kenaf. It is used as vegetable, blood tonic and remedy for liver diseases and treatment of diabetes in folk medicine. Earlier studies indicate its antioxidant activity in the protection of cell membrane integrity from the effect of oxidant (Agbor *et al.*, 2005; James and Goje, 2010). Whereas, *Murraya koenigii* (curry), apart of being a good appetizer and flavor to food, it has been implicated to have influence on lipid metabolism. Studies have shown that using the plant *Murraya koenigii* significantly lower serum total cholesterol, LDL and VLDL cholesterol and triglyceride level (Khan *et al.*, 1996).

Since these plants (*Hibiscus cannabinus* and *Murraya koenigii*) are consume as part of food materials in most Nigerian diet and the traditional method of preparing herbal remedy are mainly aqueous, this present studies is to ascertain the effect of the aqueous leaves extracts on some hematological indices, serum enzymes, serum glucose, triglyceride and cholesterol levels.

MATERIALS AND METHOD
Material
Chemical and reagents used where of analytical grade; and equipment used where all provided in the Department of Biochemistry, Kaduna State University, Kaduna.

Collection of Plant Material and Aqueous Extract Preparation
Fresh leaves of *H. cannabinus* and *M. koenigii* used where obtained from Ungwa Romi and Ungwa Rimi area of Kaduna South and North respectively during the raining season (August – October). The leaves where carefully picked from the stems and sundried for four day before pulverized to powder and stored in plastic for use.

Fifty gram of the powdered leaves of *H. cannabinus* and *M. koenigii* where separately transferred into a 500ml beaker and soaked with 300ml warm distilled water respectively, with a constant stirring for 1hr using a magnetic stirrer. On cooling, the extract where filtered with the aid of muslin cloth and re-filtered with the aid of a cotton wool plugged to a funnel to obtain a near residue free extract and stored in plastic bottle in the refrigerator. The concentrations of the extracts were noted.

Experimental Design
Twenty four albino rats where purchase from National Institute of Trypansomiasis and Onchocerciasis Research (NITOR), Kaduna Nigeria. The rats where grouped into six with four rats in each group. The control group was administered only to distilled water and palleted feed *ad libitum*. The treated group CR1 and CR2 had access to feed and a daily supply into their drinking water 5mg and 10mg/Kg body weight dose of *Murrraya koenigii* respectively. While H1 and H2 treated group had their water also treated with a dose of 5mg and 10mg/Kg body

weight of *Hibiscus cannabinus* leave extract respectively. Group ME had a mixture of *H. cannabinus* and *M. koenigii* leaves extract in their daily supplies of 5mg/Kg body weight in their drinking water. All treated group had access to feed *ad libitum* and extract for a period of seven days.

Blood Sample Collection
Blood samples were obtained via the heart with the aid of needle and syringe. Before blood sample collection rats were anesthetized with the aid of chloroform; follow by a deep surgical exposure of the heart and 5 ml of blood collected form the ventricle using a 19G needle. Two ml of blood sample for haematological studies were transferred into an EDTA bottles. While the remaining blood sample were transferred into another test tube and incubated in an upright position at room temperature for 30 min to allow clothing for serum preparation. The clothed blood was centrifuge at 1000 g for 15 min, after which the supernatant (serum) was aspirated into a sample vial and stored in the refrigerator until use (Williams, 1976).

Haematological Tests
The effects of the leaves extract of *H. cannabinus* and *M. koenigii* on the haematological indices of the rats treated group compared to control were determined. The following tests were conducted as describe by Ochei and Kolhatkar (2007), Hematocrit or Packed cell volume (PCV) and White blood cell count (WBC); estimation of haemoglobin concentration by cyanomethaemoglobin (Drabkin and Austin, 1932) method and Mean corpuscular haemoglobin concentration (MCHC) (Linne and Ringsurd, 1979; Raphael, 1983).

Estimation of Serum Glucose by Glucose Oxidase method
Serum glucose estimation was determined by the enzymatic method using the reagents kits following manufacturer's instructions (Randox Ltd, 2009).

Estimation of Serum Triacylglycerol by Glycerol Phosphate Oxidase method
Serum triacylglycerol was estimated by the enzymatic method using kits as produce and describe by Agape Diagnostic laboratory (2009).

Estimation of Serum Cholesterol Levels by Enzymatic method
Serum Cholesterol levels was determined by kits as produce and describe by Randox Ltd.

Liver Function Test
The status of the rats' liver was determined by the activities of enzymes Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) whose levels where estimated by Reitman and Frankel (1957) method. The estimation of Alanine aminotransferase activities was done by monitoring the concentration of pyruvate hydrazones formed with 2,4-dinitrophenyl hydrazine (DNPH). While AST was estimation by monitoring the concentration of oxaloacetate hydrazine formed with DNPH.

From the test samples 100 µl of the serum was transferred each into separate test tubes and to standard test tube 100 µl of standard solution (containing 2 mmol of pyruvate in distilled water) and for the blank test tube 100 µl of distilled water was placed. To all AST test tubes, 50 µl of AST substrate buffer (consisting of 200 mmol L-Aspartic and 2 mmol α-Ketoglutarate in phosphate buffer pH 7.4) was added, mixed and incubated for 30 minutes. Also, to all ALT test, 50 µl of ALT substrate buffer (consisting of 200 mmol L-Alanine and 2 mmol α-Ketoglutarate in phosphate buffer pH 7.4) was added, mixed and incubated for 30 minutes. After 30 minutes, to each test tube, 50 µl of DNPH solution was added, mixed and allowed to stand for 20 minute at room temperature. Then 5 ml of 0.4 M Sodium hydroxide solution was added to each tube, so as to stop the reaction and for colour development. The absorbance was read at 540 nm against a blank (Raphael, 1983) . Enzyme activity (IU/L) was calculated thus:

$$AST \text{ or } ALT \text{ (IU/L)} = \frac{\text{Absorbance of Test} \times \text{Conc. Of Standard}}{\text{Absorbance standard}}$$

Statistical Analysis
Means results so obtained were subjected to simple student t-test analysis at P<0.05.

RESULTS
On extraction the aqueous leave extract of *H. cannabinus* and *M. koenigii* gave a concentration of 3.06g/300ml (10.20mg/ml) and 9.08g/300ml (30.27mg/ml) respectively. The phytochemical screening of the leaves extract both gave positive results for the presence of Glycosides including; Flavonoid, Saponins, Tannin and Alkaloids such as quinine, codeine. Weight gain for all tested group increase significantly (P<0.05); while there was no significant effect of both *H. cannabinus* and *M. koenigii* extract on the determined haematological indices, serum glucose AST and ALT. However, a significant difference (P<0.05) was observed on the levels of the serum triaclyglycerol and cholesterol.

Table 1: Phytochemical Screening of Aqueous Leave Extract of *H. cannabinus* and *M. koenigii*

Phytochemical Screen	Test	Inference	
		H. cannabinus	*M. koenigii*
Glycosides			
General test	Fehling's test	+	+
Flavonoids	NaOH test	+	+
Saponins	Frothing test	+	+
Tannins	Ferric Chloride test	+	+
Alkaloids			
General test	Mayer's test	+	+
Theobromine		-	-
Quinine	Erythroquinne	+	+
Theophyine		-	-
Codeine	KFeCN test	+	+
Ergonovine	$FeCl_3$ test	-	-

Table 2: Weight Change Before and After the Administration of Rats with Feed and Aqueous Plant Extract of *H. cannabinus* and *M. koenigii*

Parameters	Control	CR$_1$	CR$_2$	H$_1$	H$_2$	ME
Initial weight (g)	110.45±7.02	100.98±5.02	100.88±5.04	92.15±15.45	120.57±16.01	106.16±17.06
Final Weight (g)	137.48±12.96^s	146.07±8.41^s	129.30±10.89^s	135.88±17.30^s	154.15±24.85^s	134.51±23.61^s

NB: Values are Mean±Standard Deviation for four rats in each group
^{S}Significant deference before treatment ($P<0.05$) (paired t-test)
CR1: Group treated with daily supply into their drinking water 5 mg/Kg body weight dose of *Murrraya koenigii*
CR2: Group treated with daily supply into their drinking water 10 mg/Kg body weight dose of *Murrraya koenigii*
H1: Group treated with daily supply into their drinking water 5 mg/Kg body weight dose of *Hibiscus cannabinus* leave extract
H2: Group treated with daily supply into their drinking water 10 mg/Kg body weight dose of *Hibiscus cannabinus* leave extract
ME: Group treated with a mixture of 5 mg/Kg body weight *H. cannabinus* and *M. koenigii* leaves extract in daily of drinking water

Table 3: Effect of *H. cannabinus* and *M. koenigii* on Some Haematological Indices and Serum Enzymes as Determined in Rats

Parameters	Control	CR$_1$	CR$_2$	H$_1$	H$_2$	ME
PCV (%)	34.33±5.51	30.00±5.89^N	31.50±5.20^N	34.50±3.87^N	31.75±0.96^N	35.00±4.40^N
WBC (gx10^9/l)	5.80±0.82	5.73±0.44^N	6.23±0.38^N	5.88±0.81^N	5.73±048^N	5.90±0.77^N
[Hb] (g/dl)	11.75±1.63	10.00±1.95^N	10.53±1.73^N	11.50±1.29^N	10.58±0.34^N	11.65±1.46^N
MCHC	33.77±0.76^N	33.34±0.09^N	33.42±0.06^N	33.33±0.00^N	33.31±0.01^N	33.29±0.05^N
AST (IU)	89.07±13.63	84.03±33.75^N	107.03±19.73^N	106.2±93.25^N	194.9±186.25^N	115.39.68.05^N
ALT (IU)	21.46±6.68	28.98±11.21^N	27.64±12.25^N	17.5±9.23^N	57.65±62.32^N	34.99±16.28^N

NB: Values are Mean±Standard Deviation for four rats in each group
^{N}Non-significant deference from the control ($P<0.05$) (paired t-test)
CR1: Group treated with daily supply into their drinking water 5 mg/Kg body weight dose of *Murrraya koenigii*
CR2: Group treated with daily supply into their drinking water 10 mg/Kg body weight dose of *Murrraya koenigii*
H1: Group treated with daily supply into their drinking water 5 mg/Kg body weight dose of *Hibiscus cannabinus* leave extract
H2: Group treated with daily supply into their drinking water 10 mg/Kg body weight dose of *Hibiscus cannabinus* leave extract
ME: Group treated with a mixture of 5 mg/Kg body weight *H. cannabinus* and *M. koenigii* leaves extract in daily of drinking water

Table 4: Effect of *H. cannabinus* and *M. koenigii* Aqueous Leave Extract on Rat Serum Glucose, Triacylglycerol and holesterol Levels

Parameters	Control	CR$_1$	CR$_2$	H$_1$	H$_2$	ME
Serum Glucose (mmol/dl)	3.88±0.83	3.68±1.36	3.41±0.80	4.27±1.37	3.44±0.37	3.16±0.60
Serum Triacyglycero (mg/dl)	65±10.83	61.68±12.84	42.18±14[S]	40.16±15.84[s]	31.34±10.53[s]	32.41±0.02[s]
Serum Cholesterol (mg/dl)	77.83±6.56	52.04±3.97[s]	50.9±9.7[s]	24.08±2.95[s]	26.82±8.05[s]	29.1±5.43[s]

NB: Values are Mean±Standard Deviation for four rats in each group
[S]Significant deference from the control (P<0.05) (paired t-test)

CR1: Group treated with daily supply into their drinking water 5 mg/Kg body weight dose
    of *Murrraya koenigii*
CR2: Group treated with daily supply into their drinking water 10 mg/Kg body weight
    dose of *Murrraya koenigii*
H1: Group treated with daily supply into their drinking water 5 mg/Kg body weight dose
    of *Hibiscus cannabinus* leave extract
H2: Group treated with daily supply into their drinking water 10 mg/Kg body weight dose
    of *Hibiscus cannabinus* leave extract
ME: Group treated with a mixture of 5 mg/Kg body weight *H. cannabinus* and *M. koenigii*
    leaves extract in daily of drinking water

DISCUSSION

The weight gain seen in this study could suggest that the plant extract increase the appetite for increase food intake ultimately leading to increase in the weight observe, as *Murraya koenigii* and *Hibiscus cannabinus* are known to be good appetizer and flavor (Afonne *et al.*, 2002; Orisakwe *et al.*, 2003 and Khan *et al.*, 1996). The presence of flavonoid and morphine (codeine) seen in *H. cannabinus* and *M. koenigii* leaves extract suggest the plants to have effect as stimulant, anti-dysenteric; since morphine is known to act as laxative and analgestic (Gillies, *et al*, 1986). Flavonoids tend to modulate the activity of enzyme, exhibit free radical scavenging activity, chelate certain metal cations, possesses antioxidant properties, enhance the resistance of low density lipoprotein (LDL) to oxidation, protect against peroxidative membrane damage and affect cellular protein phosphorylation (Adegunloye *et al.*, 1996). At the concentration of 5 and 10 mg/Kg body weight the plants extracts has no effect and did not alter haematological parameters of the rat. Moreover, the plants have been query to be a good source of blood tonic and have been claimed to be used in the treatment of anaemia (Tembhurne and Sakarkar, 2009; Okwu and Josiah, 2006). While under normal condition, the body can generate new blood cells to replace lost once (Agbor *et al.*, 2004). Although the effect of the serum glucose levels of the extract was not significant at both concentration of 5 and 10 mg/dl, but Lawal *et al* (2008) shows that at a higher concentration of 100, 150 and 200 mg/Kg, *M. koenigii* exert a significant reduction of plasma glucose level on normal and in alloxan induced diabetes in rats. Although, he said this anti-diabetic properties is less when compare with standard drugs such as chloropamide, hence cannot be used as a substituted for conventional anti-diabetes drugs.

The plant extract show a significant reduction (P< 0.05) of the triglyceride and cholesterol as low as 31.34±10.53 and 24.08±2.95 mg/dl respectively for *H. cannabinus* leaf extract. While the mixed extract of *H. cannabinus* and *M. koenigii* at 10mg/Kg gave a significant effect on the level of rat serum triglyceride and cholesterol of 32.41±0.02 and 29.1±5.43 respectively. This result suggest that the leaves extract of *H. cannabinus* and *M. koenigii* indicate the present of anti-cholesteramia and hypolipidemic properties, which also confirmed the work of Lawal *et al.* (2008); Dineshkumar, et al (2010) and Khan *et al.*, 1996. It is well established that diabetes often accompanied by lipid abnormalities, which contribute significantly to cardiovascular morbidity and mortality in diabetic patients (Kesaria *et al*, 2007). Since, lipid abnormalities accompanying with atherosclerosis is the major cause of cardiovascular disease in diabetes. Therefore ideal treatment of diabetes, in addition to glycemic control, should have a favorable effect on lipid profiles. The abnormalities in lipid metabolism lead to elevation in the levels of serum lipid and lipoprotein that in turn play an important role in occurrence of premature and severe atherosclerosis, which affects patients with diabetes (Dineshkumar *et al.*, 2010). Hence the use of this plant is suggested as adjuvant to other drugs especially as the plant is used commonly in food preparation and as vegetables and spices.

CONCLUSION

The aqueous leaf extract of *Hibiscus cannabinus* and *Murraya koenigii* exhibited significant hypolipideamic activity in normal rats. From the phytochemical analysis it was found that the major chemical constituents of the leaf extract were flavonoids and glycosides. On the basis of above evidence it is possible that the presence of flavonoids or glycosides may be responsible for the observed hypolipidemic activity. It is important to subject the plant to further pharmacological and biochemical

investigations so as to find out the active constituents responsible for hypolipidemic activity and to elucidate its mechanism of action.

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