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HYPOCHOLESTEROLEMIC POTENTIAL OF *Momordica charantia* Butanol FRACTION ON ALBINO RATS FED HIGH FAT DIET

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ABSTRACT
Hypercholesterolemia is characterized by high blood cholesterol. It is a leading cause of disease burden accounting for one-third of ischemic heart diseases and one-fifth of stroke and reports reveal a rising prevalence across many low- income countries including Nigeria. While the quest for a therapeutic remedy is ongoing, this study investigated the effect of Momordica charantia butanol fraction (MCB) on lipid profile (TC, TG, LDL-c and HDL-c) of hypercholesterolemic and normolipidemic albino rats. Crude extract of M. charantia was fractionated by liquid-liquid extraction. Acute toxicity (LD_{50}) of MCB was determined. The components of the MCB were examined by Gas Chromatography-Mass Spectrometry (GC-MS). Biochemical analyses of serum lipids were performed by reflectance photometry. Twenty-eight albino rats were divided into seven groups of four rats each. Group 1 (Control) fed on basal diet (BD). Group 2 fed on high fat diet (HFD). Group 3 and 4 fed on HFD and orally administered MCB at doses of 50 and 100 mg/kg bw/day, respectively. Group 5 fed on HFD and administered 100mg/kg of Atorvastatin. Group 6 and 7 fed on BD and administered MCB at doses of 50 and 100 mg/kg respectively. The LD_{50} of MCB was found to be ≥ 3807.89mg/kg. All treated groups showed a significant (P< 0.05) suppression of body weight compared to control and untreated HFD group (group 2). Also, a significant (P<0.05) decrease in the levels of TC, TG and LDL-c was observed in all treated high fat diet groups (group 3, 4 and 5) compared to group 2. However, HDL-c levels of treated HFD groups significantly (P<0.05) increased compared to group 2. No significant difference (P>0.05) in the level of all lipid profile parameters of treated basal diet groups (group 6 and 7) compared to the control. These findings illustrated that MCB possess hypocholesterolemic potential and may be valuable for preventing hypercholesterolemia induced by high fat diet.
Keywords: Momordica charantia, Hypercholesterolemia, Butanol Fraction

INTRODUCTION

Hypercholesterolemia refers to a metabolic disorder characterized by an increase in the concentration of plasma cholesterol (above 200mg/dL). It can be classified as primary when it is associated with congenital problems or improper food habits, or as secondary when associated with some disease such as diabetes mellitus. Previous studies have already highlighted the prevalence of hypercholesterolemia that could ultimately affect the majority of the adult population of developed countries (Kuklina *et al*., 2009). It is a leading cause of disease burden accounting for one-third of ischemic heart diseases and one-fifth of stroke and report reveal a rising prevalence across many low- income countries including Nigeria with quarter of adult affected (WHO, 2017). The rising burden of hypercholesterolemia in Nigeria corresponds with increasing rates of unhealthy diets and life styles in the population (Adeloye *et al*., 2020). Elevated level of low-density lipoprotein cholesterol (LDL) is known to predispose to cardiovascular diseases. In addition, a reduced blood level of high-density lipoprotein (HDL, <40mg/dL) cholesterol is also a critical risk factor of hypercholesterolemia-related cardiovascular diseases (CVDs) (Stone *et al*., 2014). Several studies have demonstrated that high fat or high-calorie diets can induce obesity and hyperlipidemia in the normal rodent model (Sharma *et al*., 2015; Lai *et al*., 2016). Furthermore, various clinical studies have shown strong correlations between elevated circulating triglyceride (TG), total cholesterol (TC), and reduced HDL levels as major predictors of obesity, diabetes, and hyperlipidemia (Couillard *et al*., 2005; Van Der Made *et al*., 2015).

The most commonly used pharmacological therapy for hypercholesterolemia is statins. These drugs competitively inhibit HMG CoA reductase, the regulatory enzyme of cholesterol biosynthesis. However, high-dose of statin therapy is associated with adverse effects, such as muscle pain and hepatic abnormalities (Parker *et al.,* 2013). Natural remedies from medicinal plants are therefore preferred for their affordability and fewer side effects.

Lipid-lowering medicinal plants may reduce hyperlipidemia, preventing atherosclerosis and vascular endothelial damage due to their potential of targeting multiple steps involved in its pathogenesis, with fewer side effects (Nawwar *et al.,* 2015). Present curiosity in traditional medicine has led to the exploration and development of many herbal drugs for the management of atherosclerosis (Kajal *et al.,* 2016) such as galabrol from *Glycerrhiza galabra* (Choi *et al.,* 2007) and curcumin from *Curcuma longer* (Yuan *et al.,* 2008).

Momordica charantia is both nutritious and medicinal plant belonging to the *Cucurbitaceae* family. It is commonly known as bitter melon (English) and "Garafuni" (Hausa). The combination of medicinal and vegetable usage has made *M. charantia* popular for thousands of years (Jia *et al.,* 2017). The plant is distributed widely throughout tropical and subtropical regions and considered native to the African and Australian continents (Englberger, 2009). In addition to being used as nutritious food, the plant is also well-known in African, Ayurveda and Chinese traditional systems of medicine for its use in the treatment of diabetes mellitus (Englberger, 2009). Researches on *M. charantia* has been carried out to determine its antidiabetic potential, however, most studies perform on the plant focused on crude extract of the plant. Therefore, there is need to explore the hypocholesterolemic potential of butanol fraction of this plant. The present study investigated the effect of oral administration of *M. charantia* butanol (MCB) fraction on serum lipid profile of hypercholesterolemic and normolipidemic albino rats.

MATERIALS AND METHODS

Collection and Identification of Plant

Fresh leaves and vines of *M. charantia* were obtained from Bayero University old site during the rainy season. The plant was identified and authenticated at the Herbarium Unit of the Department of Plant Biology, Bayero University Kano and given an authentication number; BUKHAN 645.

Preparation and Extraction of *Momordica charantia*

The leaves and vines of the plant were shade dried and then powdered using mortar and pestle. The powder was sieved to yield a finer powder and weighed.

Ultrasound-Assisted Extraction (UAE)

Ultrasound assisted extraction was carried out using an ultrasonicator (GT sonic machine). The procedure uses ultrasound ranging from 20 kHz to 2000 kHz (Azwanida, 2015).

The Ultrasonic machine was filled with appropriate amount of water and preheated to 40^{o}C. To each beaker containing 20g of *M. charantia* powder, 250 ml of 85% ethanol was added. The mixture was stirred and placed in the ultrasonic machine that was preset for 1 hour run time. After the run, the mixture in the beakers was filtered using a sieve with a very small mesh size <1mm. The green-colored filtrate was further filtered with Whatman filter paper and the resulting filtrate was concentrated by rotary evaporation then freeze dried.

Fractionation of the crude extract

The *M. charantia* butanol fraction (MCB) was obtained using the method of Li *et al.* (2015) with slight modification. *Momordica charantia* crude extract was dissolved in appropriate amount of water in separatory funnel. Equivalent amount of n-hexane was added into the funnel and closed. The mixture was shaken vigorously and the funnel was allowed to stand. The content separates into two layers after sometime. The bottom layer was collected and rewashed with n-hexane by similar method to completely defat the sample. The residual water-soluble fraction was further extracted twice with ethyl acetate to yield the ethyl acetate fraction. The residual water-soluble fraction was further extracted twice with butanol and allowed to stand overnight to yield water-soluble fraction and butanol fraction. The butanol fraction was concentrated by rotary evaporation and freeze dried.

Acute Toxicity Study (LD_{50})

The method of Lorke (1983) was used in the LD_{50} determination with 12 albino rats. The method involves two phases. In phase 1 three groups each containing three albino rats, were orally administered MBC fraction at doses of 10mg/kg, 100mg/kg and 1000mg/kg body weight and were observed for 24hours. In phase 2, another three groups each were containing one albino rat were orally administered MBC fraction at a dose of 1600mg/kg, 2900mg/kg, 5000mg/kg. They were observed for death and behavioral changes within 24hours. LD_{50} value was then determined using the formula.

LD50 = $\sqrt{(D_0 \times D_{100})}$. Where D_0 = Highest dose that gave no mortality and D_{100} = Lowest dose that produced mortality

Experimental Design

Twenty-eight albino rats were divided into seven groups of four rats each. Group 1 (Control) fed on basal diet (BD). Group 2 fed on high fat diet (HFD). Group 3 and 4 fed on HFD and orally administered MCB at doses of 50 and 100 mg/kg bw/day respectively. Group 5 fed on HFD and administered 100mg/kg bw of Atorvastatin. Group 6 and 7 fed on BD and administered MCB at doses of 50 and 100 mg/kg bw respectively. The treatment lasted for a period of 28 days.

Collection of Blood Sample

After four weeks of experimental period, the animals were fasted for 12 hours and all rats were sacrificed by cervical dislocation under ether anesthesia. The blood samples were collected and centrifuged (4,000 rpm/min, at 4°C for 15 minutes). The plasma was collected and lipid profile parameters were determined.

Lipid Profile Test

Lipid panel test strip (PTS) system was used to measure lipid parameters. The system uses lipid panel test strips to measure TC, HDL and TG in whole blood/plasma/serum. The LDL cholesterol is calculated from the result obtained for other parameters using the formula; {LDL = TC – HDL– (Trig/5)}. CardioChek Plus professional analyzer provides the quantitative result. This method is based on the earlier reports by Dos Santos *et al.* (2015).

A memo chip that matches the lot number on the test strip vial was inserted in to the analyzer and the analyzer was turned on. The test strip was inserted in to its slot on the analyzer. The command "apply sample" displayed on the screen of the analyzer. A capillary tube was used to apply 35-40 µL of serum on the test strip blood application window. When enough sample went into the application window the response "testing" displayed on the screen of the analyzer. Within 90 seconds the result appeared on the display screen of the analyzer.

Statistical Analysis

All data were collected and statistically analyzed using SPSS18.0. The results were expressed as mean ± standard deviation. Significance differences between group means ware analyzed with one-way ANOVA followed by Duncan multiple comparison test at $P<0.05$

RESULTS AND DISCUSSION

The yield of the crude extract obtained is 615.5g which is 30.77% of the starting sample (2000g). The yield of MCB fraction is 23.4g which is 1.17% of the starting sample used (2000g).

The result of LD_{50} study of MCB is shown in Table1. Mortality was observed only at the highest dose, 5000mg/kg (in phase two). Animal administered highest dose showed some behavioral changes (decreased appetite) and died 23 hours after administration. The LD_{50} was found to be $\geq$3807.89mg/kg using $LD_{50} = \sqrt{(D_0 \times D_{100})}$. Reports by Umokoro and Ashorobi (2006) showed that, administration of 1 to 8 g/kg of crude leave extract of *M. charantia* to albino rats is safe.

Table 2 shows that the initial body weights of the albino rats in all groups were statistically not different ($P>0.05$). The final body weight of albino rats in the HFD group (138.5±1.29 g) was 23.39% higher compared to animals in the control group (112.25±0.96 g, $P<0.05$).

There was no significant difference ($P>0.05$) between control and atorvastatin treated group. The final body weights of both MCB-treated HFD groups (HFD+MCB50 and HFD+MCB100) were significantly lower ($P<0.05$) than untreated HFD group in a dose dependent manner. Also, the final body weights of basal diet groups (BD+MCB50 and BD+MCB100) treated with MCB were significantly lower than that of the control ($P<0.05$) in a dose dependent manner. These findings agree with that of Chen *et al.* (2003) who demonstrated that *M. charantia* juice reduced weight gain without affecting energy intake or apparent fat absorption. He *et al.* (2018) illustrated the suppression of body weight by *M. charantia* leave extract in mice fed HFD. Atorvastatin was shown also to suppress body weight gain of high cholesterol diet fed mice to that of the control by Khan *et al.* (2015).

Table 3 shows lipid profile result. The untreated HFD group has significantly high ($P<0.05$) level of TC, TG, LDL-c and TC/HDL ratio while a significant decreased level ($P<0.05$) of HDL compared to the control. Plasma TC of untreated HFD group was significantly higher (172.00±9.63 mg/dL) compared to the control (117.25±8.92 mg/dL; $P<0.05$). Total cholesterol of the HFD groups treated with MCB and atorvastatin (HFD+MCB50, 145.25±13.91 mg/dL; HFD+MCB100, 128.75±7.80 mg/dL and HFD+AVS100, 120.50±10.25 mg/dL) were significantly lower ($P<0.05$) than HFD group. No significant ($P>0.05$) difference in TC level of control and atorvastatin-treated group (HFD+AVS100). Also no statistical difference ($P>0.05$) in TC level of atorvastatin treated group (HFD+AVS100) compared to HFD+MCB100 (group 4) that receives equivalent dose of MCB (100mg/kg).

The TC level of basal diet groups treated with MCB (BD+MCB50, 112.25±11.70 mg/dL; BD+MCB100, 104.75±8.22 mg/dL) were statistically similar (P>0.05) compared to the control (117.25±8.92).

Triacylglycerol (TG) level of the HFD group was significantly higher (120.25±8.14 mg/dL) than that of the control group (78.75±13.18 mg/dL; P<0.05). Treated HFD groups (HFD+MCB50, 89.25±8.92 mg/dL; HFD+MCB100, 85.50±4.51 mg/dL) has a significantly lower TG levels (P<0.05) compared to untreated HFD group. There was no statistical difference (P>0.05) between atorvastatin-treated (HFD+AVS100) group and control. Also no statistical difference (P>0.05) in the levels of TG between treated BD groups (BD+MCB50, 72.00±11.17 mg/dL and BD+MCB100, 85.5±4.51 mg/dL) and control.

The level of HDL-c of HFD group (54.75±6.80mg/dL) was significantly lower (P<0.05) than that of the control (69.5±6.61 mg/dL). All other groups showed no statistical difference (P<0.05) to control but were significantly higher than HFD group (P<0.05).

Level of LDL-c was significantly higher (P<0.05) in HFD group (93.2±15.45 mg/dL) compared to control (32±11.23 mg/dL). All the treated HFD groups (HFD+MCS50, 52.40±7.07 mg/dL; HFD+MCB100, 33.90±6.81 mg/dL and HFD+AVS100, 24.75±15.94 mg/dL) showed a significantly lower (P<0.05) LDL-c compared to untreated HFD group (group 2). No statistical differences (P>0.05) in LDL level of treated basal diet groups and the control.

Cholesterol ratio (TC/HDL) was significantly higher (P<0.05) in HFD group compared to control. The ratio was significantly lower in all treated HFD groups compared to untreated HFD (P<0.05). No significant difference in cholesterol ratio of treated basal diet and control.

The suppression of TG, LDL-c, TC/HDL and elevation of HDL-c in high dose-treated HFD group (HFD+MCB100) is comparable to that of atorvastatin. These findings indicate that MCB could improve lipid profile and lipid metabolism. Many previous studies have already demonstrated that *M. charantia* showed antilipidemic properties in diabetic animal models (Mohammady *et al.,* 2012) and HFD-induced hyperlipidemic model (He *et al.,* 2018; Wang and Ryu, 2015). Antilipidemic properties of *M. charantia* observed in previous studies could be attributed partly to compounds soluble in butanol. After 30 days of administration of saponin-rich butanol fraction from *Camellia oleifera* to HFD groups of rats, Ye *et al.* (2013) demonstrated a decrease in blood lipids of treated groups. Four-week oral administration of *A. aspera* seed saponins produced a significant (P< 0.05) decrease of TC, TG and LDL-C and a significant increase of HDL-C level in hyperlipidemic rats (Khan *et al.,* 2015). Furthermore, present study demonstrated that the level of the all the lipid profile parameters and cholesterol ratio do not differ significantly (P>0.05) with the control in both treated basal diet groups (6 and 7). Hence MCB has good weight suppressing potential but negligible hypocholesterolemic potential in basal diet model.

Gas chromatography-mass spectrometry (GCMS) Analysis revealed the presence of compounds listed in Table 4. The listed compounds include unsaturated fatty acids, phthalic acid esters, phenolic ester and saturated fatty acids. Some of these components might have played role in the observed hypocholesterolemic potential of the MBC fraction. The chromatogram of the identified compound is shown in figure 1.

Table1. Acute toxicity of MCB fraction

		PHASE I		
Dose(mg/kg bwt)	No. of rats	No. of deaths	Survival	Mortality ratio
10	3	0	3	0/3
100	3	0	3	0/3
1000	3	0	3	0/3
		PHASE II		
1600	1	0	1	0/1
2900	1	0	1	0/1
5000	1	1	0	1/1

Table 2: Effect of Oral Administration of *M. charantia* Butanol Fraction (MCB) on Body Weight of experimental animals

Groups	Specification	Initial (g)	W 4 (final) g	%increase
1	Control	90.75±1.50	112.25±0.96^{c}	23.69
2	HFD	90.50±2.08	138.50±1.29^{f}	53.04
3	HFD+MCB50	90.00±2.16	123.50±2.38^{e}	37.22
4	HFD+MCB100	90.25±2.36	116.50±1.29^{d}	29.09
5	HFD+AVS100	90.25±0.96	113.25±0.95^{c}	25.48
6	BD+MCB 50	89.50±1.29	108.75±0.96^{b}	21.51
7	BD+MCB100	90.00±0.82	105.00±0.82^{a}	16.67

Values were presented where appropriate as mean±SD (n=4). The different letters in same column indicate statistically significant difference (P <0.05) according to Duncan's multiple range test. HFD: high-fat diet; BD; Basal diet; MCB50: *M. charantia* butanol fraction 50mg/kg; MCB100: *M. charantia* butanol fraction, 100mg/kg; AVS (Atorvastatin 100 mg/kg body weight). W4: Week 4, BW: Body weight

Table 3: Effect of Oral Administration of *M. charantia* Butanol fraction on lipid profile

Groups	Specification	TC (mg/dL)	TG (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	TC/HDL
1	Control	117.25±8.92^{ab}	78.75±13.18^{abc}	69.50±6.61^{b}	32.00±11.23^{a}	1.76±0.26^{ab}
2	HFD	172.00±9.63^{d}	120.25±8.14^{d}	54.75±6.80^{a}	93.20±15.45^{c}	3.19±0.51^{c}
3	HFD+MCB50	145.25±13.91^{c}	89.25±8.92^{c}	75.00±7.12^{b}	52.40±7.07^{b}	1.94±0.07^{b}
4	HFD+MCB100	128.75±7.80^{b}	85.50±4.51^{bc}	77.75±8.54^{b}	33.90±6.81^{a}	1.67±0.14^{ab}
5	HFD+AVS100	120.50±10.25^{ab}	83.75±7.59^{bc}	79.00±7.07^{b}	24.75±15.94^{a}	1.54±0.26^{ab}
6	BD+MCB 50	112.25±11.70^{a}	72.00±11.17^{ab}	68.50±12.66^{b}	29.35±12.96^{a}	1.57±0.43^{ab}
7	BD+MCB100	104.75±8.22^{a}	65.00±10.72^{a}	70.75±4.43^{b}	20.90±3.94^{a}	1.48±0.08^{a}

Values were presented as mean ± SD (n=4). The different letters in same column indicate statistically significant difference (P <0.05) according to Duncan's multiple range test. HFD: high-fat diet; BD: Basal diet; MCB50: *M. charantia* butanol fraction 50mg/kg; MCB100: *M. charantia* butanol fraction, 100mg/kg; AVS (Atorvastatin 100 mg/kg body weight).

Table 4: GC-MS Analysis of Butanol Fraction of *M. charantia*

Peak No	Retention time	Compound	Peak area%
1	59.449	Triphenyl phosphate	1.31
2	62.240	n-Hexadecanoic acid	37.74
3	64.068	Phthalic acid, butyl hexyl ester	3.41
4	67.316	Tetradecanoic acid	37.74
5	68.991	9,12-Octadecadienoic acid (Z,Z)-,methyl ester	1.75
6	71.580	4-Octadecenoic acid	2.39
7	72.138	Heptadecanoic acid, 16-methyl ester	2.13
8	74.372	9-Octadecenoic acid, (E)-cis-Vaccenic acid	13.41
9	77.468	Octadecenoic acid	7.67
10	79.224	Phthalic acid, di(hept-3-yl) ester	6.87

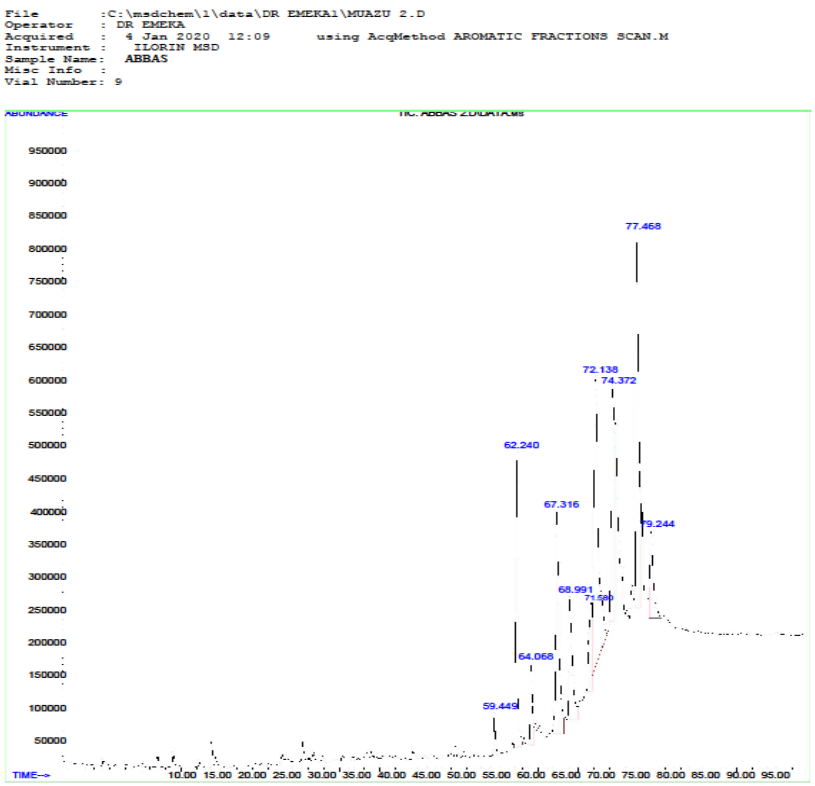

Figure 1. Chromatogram of Gas Chromatography Mass Spectrophotometry

CONCLUSION

Mormordica charantia butanol fraction has hypocholesterolemic potential in albino rats fed high fat diet evidenced by reduction of TC, TG, and LDL-c and elevation of HDL-c. Hence MCB can reduce the risk of atherosclerosis. In addition to its good hypocholesterolemic potential, MCB is also a good body weight suppressing agent in both normal and hypocholesterolemic condition. The fraction also contains components as revealed by GCMS which might have partly contributed to the observed effect.

RECOMMENDATION

It is recommended that for further work, the following should be looked into

1. Isolation and standardization of the active component of *M. charantia butanol fraction*
2. Mechanism of lipid lowering and antioxidant activity of *M. charantia butanol fraction*
3. Visceral tissue weight of the treated rats should be determined to know the effect of this fraction on organ weight

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