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Original Article

Biochemical profile of albino rats with experimentally induced metabolic syndrome fed diet formulations of *Cnidoscolus aconitifolius*, *Gongronema latifolium* and *Moringa oleifera* leaves

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Abstract

Metabolic syndrome (MS) has become a globally prevalent disease state, leading to mortality. Plants are a reservoir of compounds that have therapeutic potential and have been proven to be effective in management of a wide range of human and animal diseases. This study therefore, evaluated the effect of diet formulations of *Cnidoscolus aconitifolius* leaf (CAL), *Gongronema latifolium* leaf (GLL), and *Moringa oleifera* leaf (MOL) on some biochemical parameters of experimentally-induced MS in male albino rats. Forty-eight (48) adult male rats of 180-210 g body weight, were separated randomly into eight groups (1 – 8) comprising six rats each. Group 1 was maintained on normal diet. MS was induced in Groups 2 – 8 rats for eight weeks by high fat high carbohydrate (HFHC) diet. Afterwards, group 2 was fed normal rat diet (untreated), while groups 3 to 8 received diets formulated with GLL, CAL, MOL (100g per kg of diet) for eight weeks. Obesity indices, serum lipid profile, liver marker enzymes and antioxidant status were evaluated using standard methods. Significant ($p < 0.05$) decrease in body weight gain, total cholesterol and triacylglycerols of the treated rats were observed, while high density lipoprotein significantly ($p < 0.05$) increased compared to the untreated group. Superoxide dismutase and catalase activities significantly ($p < 0.05$) increased in the treatment groups. Treatment with the herbs showed mild Kupffer cell activation reversing periportal hepatitis induced by the HFHC diet. Results from the study indicate that CAL, GLL and MOL have therapeutic potentials that could be useful in managing components of MS.

Keywords: Antioxidant status, Lipid profile, Metabolic syndrome, High fat high Carbohydrate diet, Kupffer cells

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Introduction

Metabolic syndrome (MS), has become a major public health concern worldwide, due to increasing urbanization with its attendant influence on individuals towards surplus energy intake and sedentary lifestyle. It involves multiple metabolic pathways, having insulin resistance and central obesity as its underlying risk factors with other disorders such as: microalbuminuria, dyslipidaemia and hypertension (Grundy et al., 2005; Mamikutty et al., 2014; Roche et al., 2005). Patients with MS have five times more chances of developing type 2 diabetes, and twice more chances of mortality from cardiovascular complications than in the absence of MS (Cardinali et al., 2013; Picchi et al., 2011). MS comprises a collection of abnormalities, which notably increase the risk of cardiovascular disease and type 2 diabetes with attendant mortality (Grundy et al., 2005; Wilson et al., 2005). Included in the abnormalities are: obesity, dyslipidemia, glucose intolerance, microalbuminuria, hypertension and glucose intolerance. The most widely accepted and clinically used definition of MS was given by International Diabetes Federation (Alberti et al., 2006) as central obesity occurring alongside any two of these: high fasting blood glucose, increased blood pressure, high triacyglycerol and low high-density lipoprotein (HDL) levels.

The global prevalence of MS ranges from < 10% to about > 80%, varying based on the region, location (urban or rural area), composition of the population (gender, age, ethnicity, race), and the delineating parameters of the syndrome used in the study (Okafor, 2012). Available data show that MS affects 25% of the entire world population of adults. Differences in diet consumption, genetic factors, environment, and levels of physical activity contribute to the prevalence of MS and its various components (Bais et al., 2014). Managing MS clinically, is usually problematic as there is no singular measure or treatment for preventing or managing the syndrome holistically. Hence, successful treatment/management of MS is usually targeted at the individual components using such as lipid and glucose lowering agents, antihypertensive agents as well as insulin sensitizers (Nweje-Anyalowu et al., 2018; Okafor, 2012).

Medicinal plants have been widely used in treating individual components of MS. Increased use of herbal medicine has been documented as meeting the basic health care needs of up to 80% of those in rural areas of developing countries (Analike and Ahaneku, 2015). In addition to the presence of biological activity which is exploited in managing several diseases, use of herbs present little or no adverse effects (Saxena et al., 2013). *Gongronema latifolium* leaves (GLL) and *Moringa oleifera* leaves (MOL) possess various nutritional and medicinal values and have been used widely in treatment of disorders associated with MS. Osuagwu et al. (2013) and Balogun et al. (2016) reported the presence of phenols, tannins, alkaloids, saponins, flavonoids, phytic acids and glycosides in GLL. The anti-hypertensive, antibacterial, antifungal, antimalarial, hypolipidaemic, hypoglycaemic and antioxidant effects of GLL extracts have been documented (Mensah et al., 2008). MOL reportedly possesses antimicrobial, antidiabetic, hepatoprotective, antiobesity, hypolipidaemic and anti-hypertensive activities (Bais et al., 2014; Onwe et al., 2015). *Cnidoscolus aconitifolius* leaves (CAL) are eaten as vegetable in South Eastern Nigerian and commonly called "hospital too far" because of its various traditional medicinal claims. Phytochemical investigation of CAL as previously reported showed the presence of phenols, saponins, alkaloids, tannins and phlobatannins (Mordi and Akanji, 2012). In view of these reported properties, this study investigated the effect of diet formulations of GLL, CAL and MOL on some biochemical parameters in experimentally-induced MS using male albino rats.

Material and Methods

Animals

Forty-eight (48) adult male albino rats of 180-210 g body weight were obtained from the Faculty of Biological Sciences Animal House, University of Nigeria, Nsukka (UNN). The rats were maintained on a 12-hour dark-light cycle at room temperature; acclimatized for 14-days with *ad libitum* access to food (rat diet) and water. Ethical approval for the study regarding the use of experimental animals was obtained from the Ethics and Biosafety Committee of the Faculty of Biological Sciences, UNN with reference number UNN/FBS/EC/1028.

Collection, identification and processing of plant materials

Leaf samples of *Gongronema latifolium* and *Moringa oleifera* were obtained from Ogige Market and Obukpa respectively, both in Nsukka; while *Cnidoscolus aconitifolius* was collected from a

vegetable garden in UNN. The plants were identified and authenticated by Mr. Alfred Ozioko, a taxonomist at the Bioresources Development and Conservation Programme (BDCP) Research Centre, Nsukka, Enugu State. The plant materials were air-dried, pulverized and packaged in air-tight-polyethene bags and stored at room temperature before use.

Acute toxicity and lethality

Testing the plant leaves for acute toxicity was done using a modification of Lorke's method (Lorke, 1983). A total of 16 mice weighing 18 - 28 g were used. First, nine mice separated into three groups of three per group were orally given 10, 100, and 1000 mg/kg body weight respectively of the plant leaves in solution. The mice were observed closely for 24 h for any abnormal behaviour or lethality. Subsequently, higher doses of 1500, 2900, and 5000 mg extract/kg body weight were respectively given orally to three groups of two mice each for the 1500- and 2900- doses and three mice for 5000 mg extract/kg body weight and observed for 24 h for any abnormal behaviour or death.

Induction of metabolic syndrome

To induce MS, high-fat high-carbohydrate (HFHC) diet, made up of high fat diet and 20% fructose drinking water (FDW) was fed the experimental animals for eight (8) weeks. The high fat diet formulation method was adapted from (Picchi et al., 2011) consisting of: fat - 500 g/kg, protein - 250 g/kg, carbohydrate - 200 g/kg, fibre - 40 g/kg, vitamin and mineral mix - 10 g/kg. FDW (20 g of fructose in 100 ml of tap water) was freshly prepared at two-day intervals (Sánchez-Lozada et al., 2007; Shahraki et al., 2011) and administered *ad libitum*.

Formulation and administration of rat diets

Cnidoscolus aconitifolius leaves (CAL), *Gongronema latifolium* leaves (GLL) and *Moringa oleifera* leaves (MOL) were used in the formulation of rat diets according to Table 1 below. The plant leaves made up 10% (100g per kg) of the diet formulation for each group (Table 1). After the establishment of MS, rats were separated into eight groups with six rats in each group and fed the group specific diets for eight (8) weeks as follows:

Group 1: Commercial rat feed and tap water –Normal control
Group 2: MS rats fed commercial rat feed and tap water – Untreated control
Group 3: MS rats fed diet with CAL and tap water
Group 4: MS rats fed diet with GLL and tap water
Group 5: MS rats fed diet with MOL and tap water
Group 6: MS rats fed with combined CAL and GLL (1:1) diet and tap water
Group 7: MS rats fed with combined GLL and MOL (1:1) diet and tap water
Group 8: MS rats fed with combined CAL and MOL (1:1) diet and tap water

Samples collection

At the end of treatment duration, the animals were sacrificed after being anaesthetized using chloroform. Blood samples were drawn via cardiac puncture into non-heparinized sample tubes, allowed to clot and centrifuged at 4,000 rpm for 10 min. The serum (supernatant) was collected and stored at -20 °C for testing the various biochemical parameters. The liver of the rats was carefully excised, weighed and prepared for histopathological sectioning.

Table-1. Preparation of group-specific diet

Class of food	Source	Quantity (g/kg)
Carbohydrate	Maize	500
Protein	Processed Soya bean powder	250
Fat	Beef tallow	100
Fibre	Husk/chaff from cereals	40
Vitamin-Mineral mix	Commercially procured	10
Herbs	Plant leaves	100
Energy value (kcal/kg)		4,620

Measurement of obesity indices

Body weight of the rats was taken weekly using electronic scale. Body mass index (BMI) of the rats was taken as ratio of weight (g) and length (cm) of the rats - measured from the nasal to the anal region (Poudyal et al., 2010). The distance round the anterior abdomen was taken as abdominal circumference (AC) using a measuring tape (Mamikutty et al., 2014). The rats were anaesthetized using diethyl ether inhalation before measurements were taken. (Poudyal et al., 2010).

Determination of serum lipid profile

The serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and triacylglycerol (TAG) were determined using standard methods Trinder (1969).

Liver function tests

Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activities were determined according to Reitman and Frankel (1957). Alkaline phosphatase (ALP) and total protein levels were obtained following the method of Tietz (1995) while albumin was determined as described by Doumas et al. (1997). The methods were as outlined in the assay kits leaflets.

Oxidative stress index and antioxidant enzymes activities

Estimation of malondialdehyde (MDA) levels was carried out as described by Buege and Aust (1978). Superoxide dismutase (SOD) activity was estimated following Misra and Fridovich (1972), while catalase (CAT) activity levels were obtained according to Takahara et al. (1960).

Histopathology

The preparation of liver for histopathological examination was done according to Drury et al. (1967). A thin section of about 1-2 cm of the excised liver tissues was fixed in 10% formalin solution and incubated at 25ºC for 24 hours to ensure proper fixing. The tissue samples were dehydrated in ethanol and cleared using an automated tissue processor. Afterwards the tissues were infiltrated with paraffin wax at 50 to 52ºC, embedded into blocks, trimmed, sectioned using a microtome, mounted on clean albuminized slides, stained with haematoxylin and viewed at x400 magnification.

Statistical analysis

Statistical analysis of all data was performed using the software IBM SPSS Statistics 23. One-way ANOVA (analysis of variance) was used to test for significant differences at $p < 0.05$ while Duncan post hoc test was applied to separate means into homogenous subsets. Data is presented as mean ± SD (standard deviation).

Results

Acute toxicity

Twenty four-hour acute toxicity test of *G. latifolium*, *C. aconitifolius* and *M. oleifera* leaves on mice resulted in no death or observable indications of toxicity to 5000 mg/kg. No abnormal behaviours indicative of toxicity were observed in the mice within 24 hours after treatment.

Effect of *C. aconitifolius*, *G. latifolium* and *M. oleifera*-based diets on obesity indices of rats with experimentally-induced metabolic syndrome

The effect of diet formulations of *C. aconitifolius*, *G. latifolium* and *M. oleifera* on body weight, BMI and AC of rats with experimentally induced metabolic syndrome is shown in Tables 2, 3 and 4 respectively. Comparing with the untreated control, the rats in the treatment groups, fed the different diet formulations, had significantly ($p < 0.05$) reduced body weights (Table 2). The BMI of the diet-fed rats was significantly ($p < 0.05$) less than that of the normal control but no different ($p > 0.05$) from untreated control (Table 3). Although there was not any statistical difference ($p > 0.05$) in AC of the treated rats compared with group 2 (untreated control), it was observed that the rats administered single herbs had slightly lower AC than the untreated control while the rats administered combined herbs had slightly higher (Table 4).

Table-2. Effect of *C. aconitifolius*, *G. latifolium* and *M. oleifera*-based diets on body weight of rats with experimentally-induced metabolic syndrome

Group	Initial Body Weight (g)	Final Body Wt (g)	Body Wt Gain (g)
1. Normal	244.66 ± 10.50 ^{a}	301.66 ± 12.58 ^{a}	57.00 ± 2.64 ^{a}
2. Untreated	282.66 ± 7.02 ^{b}	381.66 ± 2.88 ^{c}	99.00 ± 9.53 ^{b}
3. CAL	290.00 ± 4.00 ^{c}	350.33 ± 2.51 ^{b}	60.33 ± 3.78 ^{a}
4. GLL	276.66 ± 6.65 ^{b}	334.33 ± 12.09 ^{b}	57.66 ± 8.62 ^{a}
5. MOL	274.00 ± 12.16 ^{b}	334.33 ± 12.09 ^{b}	60.33 ± 2.51 ^{a}
6. CAL + GLL	293.33 ± 3.21^{c}	356.66 ± 10.40^{b}	63.33 ± 8.08 ^{a}
7. GLL + MOL	283.66 ± 7.23^{b}	346.67 ± 5.68^{b}	63.00 ± 2.00 ^{a}
8. CAL + MOL	282.00 ± 6.08^{b}	341.67 ± 33.29 ^{b}	59.66 ± 22.36 ^{a}

n = 3. Values are mean ± SD

values with different alphabet superscripts in a column are statistically different at $p < 0.05$

Table-3. Effect of *C. aconitifolius*, *G. latifolium* and *M. oleifera*-based diets on body mass index (BMI) of rats with experimentally-induced metabolic syndrome

Group	Initial BMI (g/cm^2)	Final BMI (g/cm^2)	BMI Gain (g)
1. Normal	0.49 ± 0.03 [a]	0.62 ± 0.08 [b]	0.13 ± 0.06 [b]
2. Untreated	0.53 ± 0.01 [b]	0.59 ± 0.02[a]	0.06 ± 0.04 [a]
3. CAL	0.52 ± 0.02 [b]	0.56 ± 0.01[a]	0.04 ± 0.02 [a]
4. GLL	0.50 ± 0.00 [b]	0.53 ± 0.02[a]	0.03 ± 0.01 [a]
5. MOL	0.51 ± 0.02 [b]	0.53 ± 0.01[a]	0.02 ± 0.01 [a]
6. CAL + GLL	0.51 ± 0.01 [b]	0.58 ± 0.04[a]	0.07 ± 0.03 [a]
7. GLL + MOL	0.49 ± 0.01[a]	0.54 ± 0.01[a]	0.04 ± 0.02 [a]
8. CAL + MOL	0.51 ± 0.01 [b]	0.56 ± 0.01[a]	0.05 ± 0.01 [a]

n = 3. Values are mean ± SD
Values with different alphabet superscripts in a column are statistically different at $p < 0.05$

Table-4. Effect of *C. aconitifolius*, *G. latifolium* and *M. oleifera*-based diets on abdominal circumference (AC) of rats with experimentally-induced metabolic syndrome

Group	Initial AC (cm)	Final AC (cm)	AC Gain (cm)
1. Normal	13.60 ± 0.52 [a]	14.60 ± 0.36 [a]	1.00 ± 0.30 [a]
2. Untreated	15.26 ± 0.46 [b]	16.46 ± 0.25[b]	1.20 ± 0.50 [a]
3. CAL	15.90 ± 0.17 [b]	16.36 ± 0.32[b]	0.46 ± 0.15 [a]
4. GLL	15.10 ± 0.17 [b]	16.06 ± 0.11[b]	0.96 ± 0.25 [a]
5. MOL	15.86 ± 0.23 [b]	16.28 ± 0.36[b]	0.42 ± 0.30 [a]
6. CAL + GLL	16.40 ± 0.52 [b]	17.40 ± 0.79[b]	1.00 ± 0.88 [a]
7. GLL + MOL	16.16 ± 0.28[b]	16.55 ± 0.21[b]	0.38 ± 0.07 [a]
8. CAL + MOL	16.60 ± 0.05 [b]	17.20 ± 0.72[b]	1.20 ± 0.72 [a]

n = 3. Values are mean ± SD
values with different alphabet superscripts in a column are statistically different at $p < 0.05$

Effect of *C. aconitifolius*, *G. latifolium* and *M. oleifera*-based diets on serum lipid profile of rats with experimentally-induced metabolic syndrome

The rats treated GLL, MOL, CAL + GLL, GLL + MOL, and CAL + MOL had a significantly lower ($p < 0.05$) mean total cholesterol (TC) concentrations compared with group 2 - untreated control (Table 5). TC of the rats treated with MOL and CAL + GLL were significantly less ($p < 0.05$) than that of the normal control (group 1). TAG levels in all the treated except for the CAL-treated were significantly less ($p < 0.05$) than that of group 2 (untreated control). All the treatment groups had TAG levels significantly decreased ($p < 0.05$) in comparison with the group 1 (normal control) rats. Mean HDL-C levels of rats administered CAL, GLL + MOL and CAL + MOL were significantly higher ($p < 0.05$) than in group 2 - untreated control.

Table-5. Effect of *C. aconitifolius*, *G. latifolium* and *M. oleifera*-based diets on lipid profile of rats with experimentally-induced metabolic syndrome

Group	TC (mg/dl)	TAG (mg/dl)	HDL-C (mg/dl)
1. Normal	133.33 ± 13.33 [b,c]	120.27 ± 9.74 [d]	90.40 ± 13.80 [b]
2. Untreated	144.00 ± 20.13 [c,d]	101.73 ± 10.13 [c]	90.40 ± 5.2 [b]
3. CAL	162.66 ± 14.84 [d,e]	102.84 ± 1.01 [c]	141.62 ± 21.09 [c]
4. GLL	114.66 ± 7.05 [a,b]	89.82 ± 10.13 [a,b]	102.45 ± 13.13 [b]
5. MOL	96.00 ± 4.61 [a]	94.45 ± 10.70 [a,b]	51.22 ± 3.01 [a]
6. CAL+GLL	82.66 ± 5.33 [a]	70.62 ± 0.76 [a]	102.45 ± 6.02 [b]
7. GLL+MOL	136.00 ± 16.65 [b,c]	76.57 ± 4.50 [a]	117.52 ±13.80 [c]
8. CAL+MOL	122.66 ± 11.62 [a,b]	95.77 ± 10.73 [a,b]	180.80 ± 9.04 [c]

n = 3. Values are mean ± SD
Values with different alphabet superscripts in a column are statistically different at $p < 0.05$

Effect of *C. aconitifolius*, *G. latifolium* and *M. oleifera*-based diets on liver function markers of rats with experimentally-induced metabolic syndrome

Aspartate aminotransferase (AST) activity was observed to be significantly higher ($p < 0.05$) in rats given single herb diets (CAL, GLL, MOL), while that of rats treated with herbs in combination (CAL + GLL, GLL + MOL, CAL + MOL) was not significantly different compared with untreated control. No significant changes were observed in the alanine aminotransferase (ALT) activity of all the treatment groups compared with the untreated ($p > 0.05$). Alkaline phosphatase (ALP) activity showed it was significantly higher ($p < 0.05$) in the treatment groups than in the untreated. Total protein of the treated rats was not significantly changed compared with that of group 2 - untreated ($p > 0.05$). Albumin concentration was significantly lowered ($p < 0.05$) in rats that were given CAL, CAL + GLL, GLL + MOL and CAL + MOL in comparison with the controls (Table 6).

Table-6. Effect of *C. aconitifolius*, *G. latifolium* and *M. oleifera*-based diets on liver function markers of rats with experimentally-induced metabolic syndrome

Group	AST (U/L)	ALT (U/L)	ALP (U/L)	Total Protein (g/dl)	Albumin (g/dl)
1. Normal	19.89 ± 1.99 ^{a}	11.53 ± 3.15 ^{a}	7.95 ± 0.98 ^{b,c}	3.02 ± 0.70 ^{a,b}	2.57 ± 0.33 ^{b}
2. Untreated	20.21± 2.00 ^{a}	8.48 ±0.16 ^{a}	3.78 ± 0.16 ^{a}	3.68 ± 0.81 ^{a,b}	3.02 ± 0.08 ^{b}
3. CAL	34.53 ± 3.80 ^{b,c}	20.50 ± 3.08 ^{a}	5.96 ± 0.59 ^{a,b}	3.48 ± 0.08 ^{a,b}	2.49 ± 0.43 ^{a}
4. GLL	33.73 ± 3.72 ^{b,c}	10.24 ± 0.64 ^{a}	6.06 ± 0.43 ^{a,b}	3.27 ± 0.50 ^{a,b}	3.07 ± 0.76 ^{b,c}
5. MOL	37.44 ± 0.36 ^{c}	14.75 ± 4.22 ^{a}	8.90 ± 1.98 ^{c}	4.27 ± 0.75 ^{a,b}	3.07 ± 0.02 ^{b,c}
6. CAL+GLL	20.75 ± 2.81 ^{a}	15.19 ± 4.07 ^{a}	9.09 ± 0.28 ^{c}	4.18 ± 0.65 ^{a,b}	2.33 ± 0.44 ^{a}
7. GLL+MOL	22.21 ± 3.18 ^{a}	13.18 ± 4.27 ^{a}	9.46 ± 0.99 ^{c}	2.92 ± 0.43 ^{a,b}	1.90 ± 0.53 ^{a}
8. CAL+MOL	25.98 ± 0.67 ^{a,b}	20.16 ± 3.47 ^{a}	8.61 ± 0.84 ^{c}	4.85 ± 2.09 ^{b}	1.35 ± 0.16 ^{a}

n = 3. Values are mean ± SD

values with different alphabet superscripts in a column are statistically different at $p < 0.05$

Effect of *C. aconitifolius*, *G. latifolium* and *M. oleifera*-based diets on serum Malondialdehyde (MDA) and antioxidant enzymes of rats with experimentally-induced metabolic syndrome

Observed MDA concentrations of all the test groups did not statistically differ ($p > 0.05$) from the untreated. However, treatment with MOL, CAL + GLL, GLL + MOL and CAL + MOL significantly raised ($p < 0.05$) SOD activity compared with the untreated. Significantly higher ($p < 0.05$) catalase activity in all the treated groups was observed in comparison with the untreated, with the exception of the MOL- and CAL + GLL-treated groups (Table 7).

Table-7. Effect of *C. aconitifolius*, *G. latifolium* and *M. oleifera*-based diets on MDA and antioxidant enzymes of rats with experimentally-induced metabolic syndrome

Group	MDA (U/mg protein)	SOD (IU/mg protein)	CAT (IU/mg protein)
1 Normal	6.43 ± 0.76 ^{a}	74.51 ± 3.17 ^{a,b}	0.66 ± 0.10 ^{a}
2 Untreated	7.15 ± 3.16 ^{a}	42.85 ± 10.52^{a}	1.38 ± 0.73 $^{a, b}$
3 CAL	6.63 ± 2.47 ^{a}	56.97 ± 12.75 ^{a}	2.54 ± 0.06 ^{c}
4 GLL	5.19 ± 0.81 ^{a}	54.35 ± 15.58 ^{a}	1.98 ± 0.56 ^{b,c}
5 MOL	6.96 ± 4.68 ^{a}	101.24 ± 13.99 ^{b}	1.10 ± 0.32 $^{a, b}$
6 CAL+GLL	7.80 ± 0.16 ^{a}	131.88 ± 36.46 ^{b}	1.78 ± 0.54 ^{b}
7 GLL+MOL	10.89 ± 2.73 ^{a}	76.97 ± 11.29 ^{a,b}	2.47 ± 0.68 ^{c}
8 CAL+MOL	11.88 ± 5.05 ^{a}	131.16 ± 37.19 ^{b}	2.28 ± 0.64 ^{b,c}

n = 3. Values are mean ± SD

Values with different alphabet superscripts in a column are statistically different at $p < 0.05$

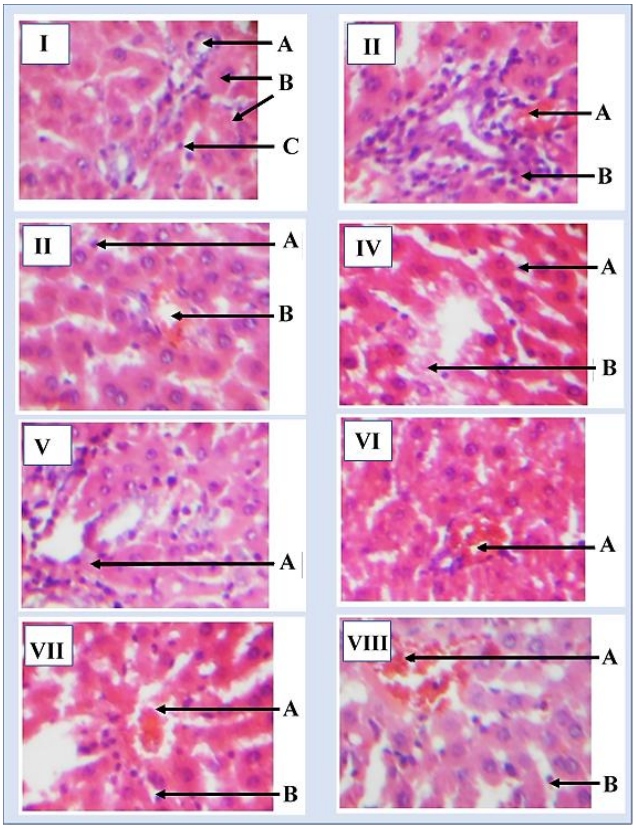

Figure-1. Liver histology of rats with experimentally-induced metabolic syndrome (H&E x 100)

Liver section photomicrographs of: I. normal control rats showing A-bile duct, B-hepatocytes and C-sinusoids; II. MS induced rats (untreated control) showing A-slight vascular congestion and B-periportal infiltration by chronic inflammatory cells; III. Rats induced and treated with CAL showing A-activated Kupffer cells and B-slight vascular congestion; IV. MS induced rats after treatment with GLL showing A-activated Kupffer cells and B-mild microvascular steatosis; V. MS induced rats treated with MOL showing A-normal liver architecture; VI. MS induced

rats treated with CAL + GLL showing A-slight vascular congestion; VII. Rats induced and treated with GLL + MOL showing A-slight vascular congestion and B-activated Kupffer cells; VIII. MS induced rats following treatment with CAL + MOL showing A-slight vascular congestion and B-activated Kupffer cells.

Effect of *C. aconitifolius*, *G. latifolium* and *M. oleifera*-based diets on liver histology rats with experimentally-induced metabolic syndrome

The liver of rats induced with MS (especially the untreated control) showed slight vascular congestion and periportal infiltration of chronic inflammatory cells (periportal hepatitis) compared to normal control which showed normal hepatic architecture. However, presence of activated Kupffer cells was evident in the CAL, GLL, CAL + MOL and GLL + MOL groups, while treatment with MOL restored normal liver architecture (Figure 1).

Discussion

Metabolic syndrome (MS) manifests with obesity as one of its important risk factors. Thus, interventions that target body weight reduction are useful in management of MS. The study findings indicate that the diet formulations of *C. aconitifolius*, *G. latifolium* and *M. oleifera* had a weight reducing effect on the rats. Weight-reducing ability of plants has been attributed to their polyphenol constituents and exploited in prevention and management of many disease conditions including obesity and related ailments (Cory et al., 2018). Thus, the high polyphenol (flavonoid) content of *C. aconitifolius*, *G. latifolium* and *M. oleifera* could be associated with the body weight-limiting effect observed. This finding agrees with that of Bakr and Header (2014) who reported a marked decrease in body weight gain after the treatment of obese rats with aqueous extracts of green tea (*Camellia sinensis*), which is known to contain appreciable quantity of polyphenols.

Treatment of MS-induced rats with diets compounded with *M. oleifera*, *G. latifolium* and *C. aconitifolius* leaves brought about a significant decrease in serum lipids (TC and TAG) indicating the potential of these herbs to help decrease the incidence of cardiovascular diseases. This is in line with previous reports on the use of *M. oleifera* and *G. latifolium* leaf extracts in the management of hyperlipidaemia (Onwe et al., 2015) indicating that the herbs could decrease the formation of triacylglycerols in the liver whilst helping in the redistribution of cholesterol. Low concentration of HDL-cholesterol predisposes to cardiovascular disease. HDL has cardio-protective properties because it serves a crucial role in mopping up excess cholesterol from peripheral tissues by reverse cholesterol transport (Ademuyiwa et al., 2005). Increased TC levels in blood is a major risk factor for coronary diseases (Ademuyiwa et al., 2005). Dyslipidaemia, mainly described as high TAG/LDL and low HDL, is an abnormal concentration of blood lipid and one risk factor for atherosclerosis which often results in hypertension, diabetes mellitus and cardiovascular disease (Hyson et al., 2002; Onwe et al., 2015). The increase observed in HDL-C in the treatment groups is in line with the findings of Ugwu et al. (2010), who studied effect of diet incorporated with *G. latifolium* and *Vernonia amygdalina* leaves on blood lipids of rats. They reported a significant decrease of serum TC and TAG and a significant HDL-C increase. Flavonoids, tannins and saponins found in some plants are reported to have hypolipidaemic activities (Ezekwe and Obidoa, 2001). Diet preparations are helpful in preventing lipid disorders which may arise as a result of metabolic disorders (Cho et al., 2002). Two processes which maintain cholesterol homeostasis are its synthesis and absorption, catalyzed by HMG-Co-A reductase in the rate limiting step. Jain et al. (2010) reported a significant decrease of HMG-Co-A reductase activity by *Moringa oleifera* extract. The groups in this study treated with GLL and CAL + GLL had the best lipid-lowering activities. As has been suggested (Ahmad-Raus et al., 2001), lipid-lowering activities might be associated with the high amounts of flavonoid and saponin contained in the studied plants. Also, the plants may possess anti-hypercholesterolaemic properties and act by preventing intestinal uptake of dietary cholesterol, preventing liver synthesis of cholesterol or by promoting its biliary secretion in faeces (Ahmad-Raus et al., 2001).

The liver enzymes AST, ALT and ALP are widespread in the tissues but are more concentrated in the liver, bone, intestine and placenta. In healthy liver, these enzymes are usually low. However, following tissue damage, changes occur in both function and membrane permeability leading to escape of enzymes from the cells (Ezeonwu and Dahiru, 2013). The liver enzymes did not reduce in the treatment groups fed diet formulations using the leaves of *Moringa*, *Gongronema* and *Cnidoscolus*. The possible explanation could be that the quantity of herbs used in

the diet formulations could not offer hepatocellular repair or any therapeutic effect to the liver damage done by the chronic consumption of high fat high carbohydrate diet. This finding is in contrast with earlier reports on the hepatoprotective effect of these plants' extracts where *Gongronema latifolium, Ocimum gratissimum* and *Moringa oleifera* leaf extracts showed hepatoprotective effects in high fat diet-induced (Das et al., 2012) and acetaminophen-induced (Ezeonwu and Dahiru, 2013) liver damage in mice and rats respectively.

Over time, research has revealed that free radicals are predisposing factors to disease conditions such as atherosclerosis, diabetes and cardiovascular disease. Free radicals can arise in obese-prone subjects from β-adrenergic receptor activation, promoting lipolysis and consequent release of free fatty acids, which in turn produce more free radicals by uncoupling mitochondrial phosphorylation (Bhandari et al., 2011). These free radicals cause oxidative damage to cell membrane lipids and proteins as well as irreversible DNA modification, in all distorting the integrity of the cell (Flora, 2007). Oxidative stress is strongly associated with damage in the body caused by free radicals (Olusi, 2002). Treatment of MS rats with diets formulated with the leaves of *M. oleifera*, *G. latifolium* and *C. aconitifolius* individually, caused a slight reduction in serum MDA. However, Ahmed et al. (2014) reported that treatment with *Moringa oleifera* extract significantly ($p < 0.05$) reduced serum MDA level in female albino rats. In our study, consumption of the plants (in combination) did not show a marked effect on MDA levels of the rats. This was rather not expected considering the high flavonoid contents of the plants. Flavonoids have several reported biological functions which include anti-inflammatory, anti-microbial and anti-tumor activities but their notable role is their ability to function as antioxidants thereby shielding the body from the debilitating actions of reactive oxygen species and free radicals. This antioxidant role is facilitated by the arrangement and composition of their chemical structure. We expected a synergistic effect resulting in marked reduction of MDA levels when the herbs were used as a combined therapy, however, this was not observed. The interaction of the varied bioactive components with each other and the anti-nutrients present in the plants may have either reduced the availability or inhibited the absorption of the particular antioxidant compounds needed.

In addition to lipid peroxidation, oxidative stress is characterized by the reduced antioxidant enzymes activities. These enzymes including CAT, SOD, GST, and GPx play active roles mopping up free radicals during oxidative stress conditions (Noeman et al., 2011). The synergistic antioxidant property of flavonoidal compounds in *M. oleifera, G. latifolium* and *C. aconitifolius* contributed to the raised SOD and catalase levels (Umar, 2012). Other studies have reported significant antioxidant property of some other plants on serum, kidney and liver SOD and catalase activities in high fat diet rat models (Umar, 2012), attributing the antioxidant properties of plants mainly to their phenolic constituents. Usoh and Akpan (2015) reported higher antioxidant activity of *G. latifolium* leaf used in combination with *Ocimum gratissimum*, pointing to a possible synergistic interaction of the bioactive constituents of these leaves.

The observed vascular congestion and inflammation in liver of rats following consumption of HFHC diet are characteristic of the metabolic disease state. Chronic inflammation, brought about in metabolic cells in response to surplus nutrients/ energy (Gregor and Hotamisligil, 2011), is seen in cases of obesity, insulin resistance, type 2 diabetes, and cardiovascular disease (Furuhashi et al., 2011). Treatment with combinations of the herbs mainly caused mild Kupffer cell activation, which is a compensatory response that serves to minimize cellular and organ damage in disease states (Dixon et al., 2013). The presence of slight vascular congestion in almost all the treatment groups suggests that the herbs did not fully repair or reverse the damage already done to the hepatocytes. This inference agrees with the results of the serum liver enzyme markers reported above. The observation of normal liver architecture in the MOL-group herein agrees with the findings of Bais et al. (2014) that methanol extract of *M. oleifera* leaf restored normal hepatocytes in obese rats.

Conclusion

The utilization of feed formulations of *Cnidoscolus aconifolius, Gongronema latifolium* and *Moringa oleifera* as treatment for rats with experimentally induced metabolic syndrome in this study showed reduction in metabolic and cardiovascular risks in terms of weight reduction, favourable lipid profile and increase in antioxidant enzymes (SOD and catalase) activities. Thus, the herbs have some therapeutic effects that could be exploited in the management of metabolic syndrome components.

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Contribution of Authors

Uchendu NO: Carried out the laboratory experiments, statistical analysis, and drafting of the manuscript.
Ezechukwu CS: Guided the research, helped in statistical analysis and edited the manuscript
Ezeanyika LUS: Conceptualized the study, guided and supervised the research