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PROTECTIVE EFFECTS OF METHANOLIC EXTRACT OF *CASSIA SIEBERENA* LEAVES AGAINST HIGH- FAT DIET- INDUCED METABOLIC SYNDROME IN RATS

Nweje-Anyalowu Paul Chukwuemeka[1]* Agatemor Uzuazokaro Mark-Maria[2], Idakwoji Precious Adejoh[2], Iserhienrhien Lucky Osafanme[3]

[1]Department of Biological Sciences, Biochemistry Section, Faculty of Science, Clifford University, Owerrinta, Abia State, Nigeria.
[2]Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria.
[3]Department of Biology, Nigeria Maritime University, Okerenkoko, Delta State, Nigeria.

***Corresponding Author: Nweje-Anyalowu Paul Chukwuemeka**
Department of Biological Sciences, Biochemistry Section, Faculty of Science, Clifford University, Owerrinta, Abia State, Nigeria.

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ABSTRACT

This study investigated the possible protective effect of methanol extract of *Cassia sieberena* leaves (MECS) against high- fat diet (HFD) induced metabolic syndrome in rats. Forty (40) albino rats were randomized into 4 groups of 10 animals each. Group 1 served as control and the rats were fed standard rat diet for 90 days. Rats in Group 2 were fed HFD for 90 days. Group 3 rats were fed HFD for 90 days but administered MECS (200 mg/kg b.w) from days 31- 90 while rats in Group 4 were fed HFD and administered MECS (200 mg/kg/day) concurrently for 90 days. The effects of treatment on some components of metabolic syndrome (obesity, hyperglycaemia and insulin resistance) were investigated through the measurement of body weight, fasting blood sugar (FBS), serum insulin level, total cholesterol (T. Chol.), triacylglyceride (TAG), High density lipoprotein (HDL) and Low density lipoprotein (LDL) concentrations. Results showed that administration of the extract for 30 and 90 days significantly ($p< 0.05$) reduced body weight, FBS, insulin level, TAG and LDL as well as significantly ($p< 0.05$) increased serum levels of HDL. It was concluded that *Cassia sieberena* leaves possess significant anti-metabolic syndrome potential. More research is required however, to find out the various mechanisms by which the extract acts on the various components of the metabolic syndrome.

KEYWORDS: *Cassia sieberena*, methanol, triacylglyceride, High density lipoprotein.

1.0 INTRODUCTION

The metabolic syndrome (MetS) is a major public-health concern and it's a consequence of urbanization, surplus energy intake, increasing obesity, and sedentary life habits. MetS confers a 5-fold increase in the risk of type 2 diabetes mellitus (T2DM) and 2-fold the risk of developing cardiovascular disease (CVD) over the next 5 to 10 years.[1] Furthermore, patients with the MetS are at 2- to 4-fold increased risk of stroke, a 3- to 4-fold increased risk of myocardial infarction (MI), and 2-fold the risk of dying from such an event compared with those without the syndrome[2] regardless of a previous history of cardiovascular events.[3]

MetS is defined by a constellation of an interconnected physiological, biochemical, clinical, and metabolic factors that directly increases the risk of atherosclerotic cardiovascular disease, Type 2 diabetes mellitus (T2DM), and all cause mortality.[4,5] This collection of unhealthy body measurements and abnormal laboratory test results include atherogenic dyslipidemia, hypertension, glucose intolerance, proinflammatory state, and a prothrombotic state. There have been several definitions of MetS, but the most commonly used criteria for definition at present are from the World Health Organization (WHO).[6]

Worldwide prevalence of MetS ranges from <10% to as much as 84%, depending on the region, urban or rural environment, composition (sex, age, race, and ethnicity) of the population studied, and the definition of the syndrome used.[7,8] In general, the IDF estimates that one-quarter of the world's adult population has the MetS.[9] Higher socioeconomic status, sedentary lifestyle, and high body mass index (BMI) were significantly associated with MetS. Cameron *et al.* have concluded that the differences in genetic background, diet, levels of physical activity, smoking, family history of diabetes, and education all influence the prevalence of them MetS and its components.[10]

MetS is a state of chronic low grade inflammation as a consequence of complex interplay between genetic and environmental factors. Insulin resistance, visceral adiposity, atherogenic dyslipidemia, endothelial dysfunction, genetic susceptibility, elevated blood pressure, hypercoagulable state, and chronic stress are the several factors which constitute the syndrome.

Clinical identification and management of patients with the MetS are important to begin efforts to adequately implement the treatments to reduce their risk of subsequent diseases.[11] Effective preventive approaches include lifestyle changes, primarily weight loss, diet, and exercise, and the treatment comprises the appropriate use of pharmacological agents to reduce the specific risk factors. Pharmacological treatment should be considered for those whose risk factors are not adequately reduced with the preventive measures and lifestyle changes.[12] The clinical management of MetS is difficult because there is no recognized method to prevent or improve the whole syndrome, the background of which is essentially insulin resistance.[13] Thus, most physicians treat each component of MetS separately, laying a particular emphasis on those components that are easily amenable to the drug treatment. In fact, it is easier to prescribe a drug to lower blood pressure, blood glucose, or triglycerides rather than initiating a long-term strategy to change people's lifestyle (exercise more and eat better) in the hope that they will ultimately lose weight and tend to have a lower blood pressure, blood glucose, and triglycerides. Traditionally, many medicinal plants have been used to treat the individual components of the metabolic syndrome. In this study we used a suitable animal model that mimics all these symptoms of human metabolic syndrome to test the potential pharmacological properties of *Cassia sieberiana* in the management of obesity, diabetes, hypertension and related metabolic disorders.

Cassia sieberena is a common tree in Senegal to Nigeria. It is also found in East Africa. The phytochemical analysis of the roots had shown the presence of flavonoids, anthracenic derivates and non hydrolysable tannins.[14] Previous studies have shown various pharmacological potentials of the plant. Ethanolic root extract of *C. sieberiana* is reported to possess antiparasitic effect, myorelaxant and antispasmodic activity.[15] It was also shown that *C. sieberiana* extracts had antimicrobial activity against *Neisseria gonorrhoeae*, *Herpes simplex virus* type I and African swine fever virus.[16] In Senegal, the aqueous root extract of *C. sieberiana* was used in traditional medicine to treat pain and inflammation.[14] Guatta[17] also showed that aqueous root extract of *C. sieberiana* possessed both analgesic and anti-inflammatory activities, which have justified their use in Senegal traditional medicine to treat pain and inflammation.

2.0 MATERIALS AND METHODS

2.1 Materials

2.1.1 Feed composition

The control diet was derived from standard rats' feeds commercially-propounded and sold by Ladokun feeds® while the high- fat diet was prepared from individual constituents.

2.1.2 Animals

Adult Albino rats were used for this study. The animals were housed in well aerated experimental animal cages and maintained under standard conditions. They were acclimatized for 7 days prior to the commencement of the study. During this period, they were fed on standard rat chow (Ladokun feeds, Nigeria Limited®) and had access to clean drinking water.

2.1.3 Chemicals and drugs

Assay kits for biochemical analyses were commercial kits from Randox Laboratories Limited, United Kingdom. All other chemicals used in this study were of analytical grade and were purchased from Sigma Chemical Co. Ltd (USA) through a local vendor.

2.2 Methods

2.2.1 Plant Collection and Identification

The leaves of *Cassia sieberiana* were collected from a natural habitat in Agbeji Area of Kogi State, Nigeria. The plants were identified at the herbarium unit of Biological Sciences Department, Ahmadu Bello University, Zaria and voucher specimens were deposited for future references.

2.2.2 Preparation of Extracts

The leaves of *Cassia sieberiana* were shade- dried for seven (7) days and pulverized using an electric blender. Two thousand (2000) gram of the pulverized leaves was cold- macerated in methanol for 72- hours. The resulting mixture was filtered using Whatmann filter paper (Size No1) and the extract was concentrated using a vacuum evaporator.

2.2.3 Acute Toxicity Study

The oral median lethal dose (LD50) of the extract was determined in rats according to the method of Lorke. [18]

2.2.4 Experimental Design

Forty (40) albino rats were randomized into 4 groups of 10 animals each and fed/ treated as follows:

Group 1: Animals were fed with a standard rat diet (30% protein, 35% carbohydrates, 25.5% fat and 9.5% crude fibre) for 90 days.

Group 2: Animals were fed with HFD (15.5% protein, 18.5% carbohydrates, 62.5% fat and 3.5% crude fibre. Fat component was derived from margarine) for 90 days.

Group 3: Animals were fed with HFD for 90 days but treated with MECS (200 mg/kg/day) from days 61- 90.

Group 4: Animals were fed with HFD and treated with MECS (200 mg/kg/day) concurrently for 90 days.

2.2.4.1 Measurement of Body Weight and BMI

Body weight and body length Body (measured as nose-anus length) were measured at the beginning and end of the study. They were used to calculate the body mass index (BMI) of the rats as follows:

Body mass index (BMI) = body weight (g)/ length^2 (cm^2).

2.2.4.2 Determination of fasting blood glucose

Fasting blood glucose was assessed by the using a glucometer (Fine Test®) with its corresponding strips. Prior to assessing fasting blood glucose, the animals were fasted overnight, but were allowed free access to water.

2.2.4.3 Lipid profile

Total cholesterol, triglycerides and HDL cholesterol were assessed in the serum of experimental animals. After the experimental period, animals were sacrificed and blood samples collected from them. The blood samples were centrifuged at 4°C for 20 min in a cold centrifuge and aliquots were then stored at -20°c until assayed.

Determination of total cholesterol

The levels of total cholesterol in the treatment groups were determined using Randox kits. A method of enzyme hydrolysis as described by[19,20] was used.

Determination of triacylglyceride concentration

This was measured by colorimetric method as described by.[21,22]

Determination of HDL – cholesterol

This was determined in the serum by the method of.[23]

Determination of HDL – cholesterol

Friedwald calculation method was used.

2.2.4.4 Plasma insulin determination

This ELISA is a solid phase two-site enzyme immunoassay as described by (Engvall and Perlmann, 1970). It is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the insulin molecule. During incubation insulin in the sample reacts with anti-insulin antibodies bound to the microtitration well and with peroxidase conjugated anti-insulin antibodies. A washing step removes unbound enzyme labeled antibody. The bound conjugate is detected by reaction with 3,3,5,5-tetramethylbenzidine (TMB). The reaction is stopped by adding acid to give a colorimetric endpoint that is read spectrophotometrically at a wavelength of 450 nm using a microplate reader. ELISA kits were procured from Sigma-Aldrich®.

2.2.5 Statistical Analysis

Statistical analysis was carried out using SPSS version 20.0. All the data were expressed as mean ± SEM and the statistical differences between the means were determined by one way analysis of variance (ANOVA) which was followed by Duncan test and difference between means at $P > 0.05$ were considered significant.

3.0 RESULTS

3.1 Acute Toxicity

The results of acute toxicity studies showed no mortality or physical changes in skin and fur, eyes and mucus membrane, respiratory rate, circulatory signs, autonomic and central nervous system effects up to a dose of 5000 mg/kg of methanol extract of *Cassia sieberena.* The oral LD_{50} of the extract was then taken to be > 5000 mg/kg.

3.2 Effect of Methanol Extract of *Cassia sieberena* Leaves on Body Weight and BMI of High- fat Diet-fed Rats

The effect of methanolic extract of *Cassia sieberena* on the body weight and BMI of high- fat diet- fed rats are shown in Tables 1 and 2 respectively. After 90 days of feeding the rats in Group 2 with the high- fat diet alone, the group showed significant ($p \leq 0.05$) increase in body weight compared to Group 1 (standard diet- fed rats). Group 3 rats administered 200 mg/ kg of the extract for 30 days also showed significant ($p \leq 0.05$) increase in body weight compared to Group 1 (standard diet- fed rats). However, Group 4 rats administered 200 mg/ kg of the extract for 90 days showed no significant ($p > 0.05$) difference in body weight compared to Group 1 (standard diet) (Table 1). After 90 days of feeding the rats in Group 2 with the high- fat diet alone, the group also showed significant ($p \leq 0.05$) increase in BMI compared to Group 1 (standard diet- fed rats). Group 3 rats administered 200 mg/ kg of the extract for 30 days and Group 4 rats administered 200 mg/ kg of the extract for 90 days showed no significant ($p > 0.05$) difference in BMI compared to Group 1 (standard diet fed rats) (Table 2).

Table 1: Effect of the Administration of Methanolic Extract of *Cassia sieberena* Leaves on Body Weight and BMI of High- fat Diet Fed Rats.

Group	Initial Body Weight (g)	Final Body Weight (g)	Body Weight Gain (g)
Group 1	100.19± 9.16^a	227.17± 12.43^a	126.98± 5.87^a
Group 2	105.41± 7.35^a	293.07± 15.15^b	187.66± 9.23^b
Group 3	102.11± 7.97^a	269.83± 12.18^b	167.72± 7.15^b
Group 4	110.18± 7.19^a	230.31± 10.63^a	120.13± 4.14^a

Data are presented as mean ± SD. Data was analysed by one- way ANOVA followed by Duncan post- hoc test for multiple comparisons, (n=10). Mean values having different lower case alphabets as superscripts are considered significant (p< 0.05) across the columns.

Group 1: Control received standard rat diet. Group 2: received high-fat diet. Group 3: received high- fat diet for 90 days + 200 mg/ kg MECS days 61- 90. Group 4 received high fat diet + 200 mg/ kg MECS for 90 days.

Table 2: Effect of the Administration of Methanolic Extract of *Cassia sieberena* Leaves on BMI of High- fat Diet Fed Rats.

Group	Initial BMI (g/ cm)	Final BMI (g/ cm)	BMI gain (g/ cm)
Group 1	0.53± 0.06[a]	0.69± 0.07[a]	0.16±0.01[a]
Group 2	0.55± 0.04[a]	0.91± 0.05[b]	0.36±0.03[b]
Group 3	0.54± 0.07[a]	0.75± 0.07[a]	0.21±0.05[a]
Group 4	0.56± 0.01[a]	0.72± 0.03[a]	0.16±0.03[a]

Data are presented as mean ± SD. Data was analysed by one- way ANOVA followed by Duncan post- hoc test for multiple comparisons, (n=10). Mean values having different lower case alphabets as superscripts are considered significant (p< 0.05) across the columns.

Group 1: Control received standard rat diet. Group 2: received high-fat diet. Group 3: received high- fat diet for 90 days + 200 mg/ kg MECS days 61- 90. Group 4 received high fat diet + 200 mg/ kg MECS for 90 days.

3.3 Effect of Methanol Extract of *Cassia sieberena* Leaves on Fasting Blood Sugar (FBS) of High- fat Diet- fed Rats

The effect of methanolic extract of *Cassia sieberena* on the FBS of high- fat diet- fed rats is shown in Figure 1. After 90 days of feeding the rats in Group 2 with the high- fat diet, the group showed significant ($p \leq 0.05$) increase in FBS compared to Group 1 (standard diet- fed rats). Group 3 rats administered 200 mg/ kg of the extract for 30 days also showed significant ($p \leq 0.05$) increase in FBS compared to Group 1 (standard diet fed rats). Group 4 rats administered 200 mg/ kg of the extract for 90 days however, showed no significant ($p > 0.05$) difference in FBS compared to Group 1 (standard diet- fed rats).

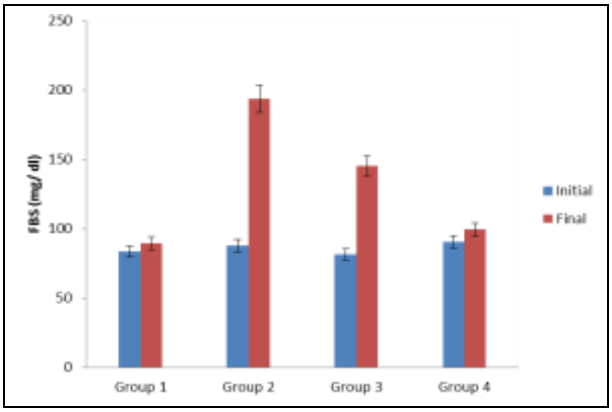

Figure 1: Effect of Methanol Extract of *Cassia sieberena* Leaves on Fasting Blood Sugar (FBS) of High- fat Diet- fed Rats.

3.4 Effect of Methanol Extract of *Cassia sieberena* Leaves on Plasma Insulin Level of High- fat Diet- fed Rats

The effect of methanolic extract of *Cassia sieberena* on the insulin level of high- fat diet- fed rats is shown in Figure 2. 90 days of feeding Group 2 rats with the high-fat diet, significantly ($p \leq 0.05$) increased the insulin level compared to Group 1 (standard diet- fed rats). Groups 3 and 4 rats administered 200 mg/ kg of the extract for 30 and 90 days respectively showed no significant ($p > 0.05$) difference in insulin level compared to Group 1 (standard diet- fed rats).

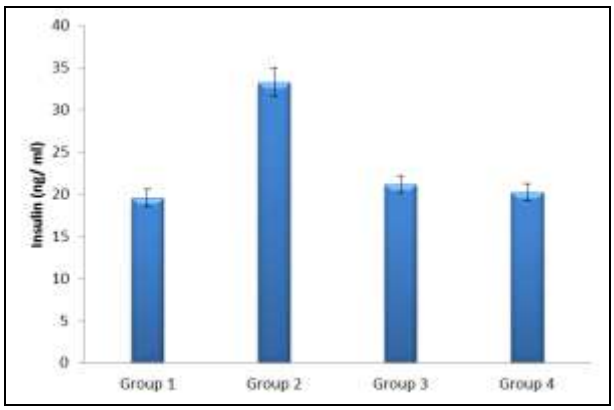

Figure 2: Effect of Methanol Extract of *Cassia sieberena* Leaves on Plasma Insulin Level of High- fat Diet- fed Rats.

Data are presented as mean ± SD. Data was analysed by one- way ANOVA followed by Duncan post- hoc test for multiple comparisons, (n=10). Mean values having different lower case alphabets as superscripts are considered significant (p< 0.05) across the columns.

Group 1: Control received standard rat diet. Group 2: received high-fat diet. Group 3: received high- fat diet for 90 days + 200 mg/ kg MECS days 61- 90. Group 4 received high fat diet + 200 mg/ kg MECS for 90 days.

3.5 Effect of the Administration of Methanolic Extract of *Cassia sieberena* Leaves on Lipid of High-fat Diet Fed Profile of Rats

The effect of methanolic extract of *Cassia sieberena* on the lipid profile of high- fat diet- fed rats is shown in Table 3. Feeding of rats in Group 2 with the high- fat diet for 90 days significantly ($p \leq 0.05$) increased total cholesterol, triacylglyceride and LDL-C concentrations with a corresponding significant ($p \leq 0.05$) decrease in HDL- C concentration compared to Group 1 (standard diet- fed rats). Group 3 rats administered 200 mg/ kg of the extract for 30 days showed significant ($p \leq 0.05$) increase in total cholesterol, triacylglyceride and LDL-C concentrations but showed no significant ($p > 0.05$) difference in the HDL- C concentration compared to Group 1 (standard diet- fed rats). Group 4 rats administered 200 mg/ kg of the extract for 90 days showed no significant ($p > 0.05$) differences in total cholesterol, triacylglyceride, HDL- C and LDL- C concentrations compared to Group 1 (standard diet- fed rats).

Table 3: Effect of the Administration of Methanolic Extract of *Cassia sieberena* Leaves on Lipid Profile of High-fat Diet Fed Rats.

Treatment	Tchol (mg/dl)	TAG (mg/dl)	HDL- C (mg/dl)	LDL- C (mg/dl)
Group 1	75.23± 3.81^{a}	69.31± 6.71^{a}	45.23± 3.17^{b}	16.16± 5.23^{a}
Group 2	108.15± 4.18^{b}	90.13± 3.38^{b}	13.81± 2.45^{a}	76.31± 6.81^{c}
Group 3	99.36± 8.70^{b}	85.37± 5.18^{b}	38.23± 5.23^{b}	44.06± 8.21^{b}
Group 4	80.13± 5.23^{a}	75.19± 3.21^{a}	40.17± 3.17^{b}	24.92± 3.20^{a}

Data are presented as mean ± SD. Data was analysed by one- way ANOVA followed by Duncan post- hoc test for multiple comparisons, (n=10). Mean values having different lower case alphabets as superscripts are considered significant ($p < 0.05$) across the columns.

Group 1: Control received standard rat diet. Group 2: received high-fat diet. Group 3: received high- fat diet for 90 days + 200 mg/ kg MECS days 61- 90. Group 4 received high fat diet + 200 mg/ kg MECS for 90 days.

4.0 DISCUSSION

MetS is defined by a constellation of an interconnected physiological, biochemical, clinical, and metabolic factors that directly increases the risk of atherosclerotic cardiovascular disease and Type 2 diabetes mellitus. [20],[21] These risk factors which include central obesity, elevated blood pressure, inflammation, impaired glucose tolerance, insulin resistance, and dyslipidemia are responsible for the increased morbidity and mortality in humans. It is therefore, important to target these established biological alterations for the treatment and reduction of clustering risk factors of this syndrome. In this study we used a suitable animal model that mimics all these symptoms of human metabolic syndrome to test the potential pharmacological properties of the methanolic extract of *Cassia sieberiana* leaves in the management of obesity, diabetes and related metabolic disorders.

The results reported here confirm that HFD does induce insulin resistance and mimics the metabolic characteristics of type 2 diabetes, with increase in blood glucose and body weight, as observed by study of Hamza.[24] Many studies have reported that HFD might be a good way to initiate the insulin resistance[25,26] which is the consequence of a number of defects including impaired insulin secretion by the pancreatic cell, resistance of peripheral tissues to the glucose utilizing effect of insulin and augmented hepatic glucose production.[27] Decreased glycolysis impeded glycogenesis and increased gluconeogenesis are some of the changes of glucose metabolism in the diabetic liver.[28]

Intake of HFD for 90 days significantly increased the body weight and BMI. After treatment of Groups 3 and 4 with 200 mg/kg of *Cassia sieberena* extract for 30 and 90 days respectively, both the body weight and BMI significantly reduced. Increase in the influx of greater amounts of non-esterified fatty acids to liver causes an increase in triacylglycerol level which may cause dyslipidemic changes in the obesity.[29] It has been demonstrated that changes in lipid concentrations and lipoprotein fractions are associated with the increased risk for obesity-related metabolic conditions. It has been found that obesity is a significant risk factor for the development of dyslipidemia.[30,31]

Results of this study revealed that administeration of 200 mg/kg/day of *Cassia sieberena* had a favorable effect on the lipid profile. It decreased TG, TC and LDL cholesterol and also increased HDL cholesterol as compared to control. The observed reduction in serum cholesterol may be attributed to the levels of polyphenolic (flavonoids, tannin and saponins) compounds present in the extract. Other studies have shown that soluble dietary fibres (SDF) in plants are known to bind to dietary cholesterol and prevent or reduce its absorption by the small intestine.[32,33] The extract produced a decrease in serum triacylglycerol level. The extract might have acted through an alteration in the level of interleukin-6 (IL-6) which mediates energy mobilization in the muscles and fat tissues.[34] An inverse proportionality exists between serum triacylglycerol level and IL-6 and TNF-α..[35] In addition, reduction in the serum cholesterol level in this study does not seem to be accompanied by lipolysis, as seen in the reduction in the serum TAG levels. The increase in the serum HDL-C level by the extract could possibly be due to an increase in the biosynthesis of HDL-C in the liver due to the presence of flavonoids in the extract.[36] With

increase in HDL- C serum level, there will be an enhanced cholesterol excretion as more of it would be transported from the peripheral tissues to the liver for excretion. The observed decrease in the serum LDL-C levels may explain the serum cholesterol-lowering capability of the extract. This effect could possibly be due to enhanced reverse cholesterol transport and bile acid excretion through inhibition of apo B production, needed for LDL-C production, transport and binding.[37] The increase and decrease in the serum HDL-C and LDL-C respectively, is beneficial as it reduces the risk of developing atherosclerosis, a vascular disease affecting blood circulation in the coronary, central, and peripheral arteries.[38]

Results of this study also revealed that administeration of 200 mg/kg/day of *Cassia sieberena* produced a significant reduction of blood glucose and insulin levels. The anti- hyperglycaemic activity of *Cassia sieberena* could possibly attributed to the relatively high antioxidant activity of its leaves. The extract might have also produced anti-hyperglycaemic activity through the release of insulin by inhibiting the ATP-sensitive potassium channels in the membrane of the residual beta-cells just like sulfonylureas and meglitinides. It is also possible that the extract might have potentiated the action of insulin to stimulate glucose uptake and utilization by tissues, especially by the liver, skeletal muscle, and adipose tissue.[39]

5.0 CONCLUSIONS

The study clearly shows that *Cassia sieberena* leaves possess significant anti-metabolic syndrome potential. The extract exerted a beneficial action on blood glucose level, serum lipids and weight gain. More research is required however, to find out the various mechanisms by which the extract acts on the various components of the metabolic syndrome.

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