

Ameliorative Effect of Methanolic Extract of *Cassia occidentalis* (MECO) Whole Plant, on Triton X-100-Induced Hyperlipidaemia in Albino Rats

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Abstract: This study revealed the evaluation of phytochemicals and ameliorative effect of methanolic whole plant extract of *Cassia occidentalis* (MECO) on triton X-100-induced hyperlipidaemia in albino rats. Qualitative phytochemical analysis of MECO was conducted using standard methods. In hyperlipidemic model, the rats were divided into five groups, where hyperlipidaemia was induced to all groups with the exception of normal control group which received only normal saline instead. On completion of 14 days of treatment with MECO and Simvastatin (Standard drug) on the hyperlipidemic rats, blood was collected through cardiac puncture for estimation of serum lipid profile with subsequent cervical dislocation of the rats. Qualitatively, MECO shows variety of phytochemicals including flavonoids, saponins, tannins, anthraquinones, cardenolides, and alkaloids. Upon administration of 250-2000 mg/kg body weight of the extracts for acute toxicity test, there was no any observable behaviour change and or mortality recorded. Both the two doses (200 and 400 mg/kg body weight) of MECO in the treatment of hyperlipidaemia show significant ($p < 0.05$) increased in serum high-density lipoprotein cholesterol (HDL-c) and decreased in total cholesterol (TC), triglycerides TG, very low-density lipoproteins (VLDL-c) as well as low-density lipoproteins (LDL-c) in test groups as compared to untreated hyperlipidemic groups. It can therefore, be concluded that MECO whole plant maintain lipid homeostasis by restoring the abnormal level of serum lipid profile back to normal and thereby minimizing the risk of coronary heart diseases and their associates in hyperlipidemic albino rats.

Keywords: Albino Rats, *Cassia occidentalis*, Hyperlipidaemia, Lipid Profile, MECO

1. Introduction

Obviously, the increase in prevalence of hyperlipidaemia is on the alarming part worldwide. Several findings have revealed the strong relationship between hyperlipidaemia Cardiovascular Diseases (CVD), and other metabolic syndromes including Obesity, Type two Diabetes mellitus to mention a few [1]. Study shows that increase in blood cholesterol level escalates the risk of more than 50% cases of CVD in various populations globally [2]. Hyperlipidaemia is characterized by elevated levels of Total Cholesterol (TC), Triglycerides (TG) Low-Density Lipoprotein cholesterol (LDL-c) and Very Low Density Cholesterol (VLDL-c) with consequent decrease in High density Lipoprotein Cholesterol (HDL-c) in blood [3]. This initiates the hardening of coronary arteries or atherosclerosis which reduce the blood flow with consequent induction of ischemia, in which if not giving urgent attention may eventually leads to heart failure [3, 4].

More so, in addition to genetics, several factors including age, diet (rich in saturated and trans-fats), sedentary life-style, alcohol consumption, hypertension and other endocrine disorders contribute immensely to the development of coronary heart diseases with eventual heart failure if not treated in the early stage [5]. Therefore, employing dietary intake regulation and engagement in routine exercise to burn down calories minimizes the risk of heart problems and keep the blood lipids within their normal levels. Despite the commercial availability of authentic lipid lowering orthodox medicines such as statins, fibrates,

simvastatin etc, for the treatment of this health hazards, some of the conventional drugs are expensive and exhibit certain side effects such as diarrhoea, nausea, myositis and abnormal liver function [5]. In order to address such issue of side effect induction, researchers are focusing on natural remedies and evaluating medicinal plants to treat hyperlipidaemia due to their cost effectiveness and minimal or no side effects [6].

Evidently, plants have been used as a traditional medicine and pharmacopoeital drugs from ancient times, and medicinal plants have been used for the treatment of illness since ancient period [7]. Numerous plant-derived therapeutic agents for the modern medicine have been provided by medicinal plants [8]. Most of the plants exhibit a variety of phytopharmaceuticals, which has important applications in the fields of agriculture, human and veterinary medicine. This plays a major role in developing novel drugs for the treatment and prevention of diseases. Therefore it is very important to have sufficient knowledge regarding herbs not only because of their widespread use, but also because they have the potentials to cause toxic reactions or interact with other drugs [9].

Although in traditional medicine *Cassia* species have been well known for their laxative and purgative properties and for the treatment of skin diseases. *Cassia occidentalis* has been used as a folklore medicine for hepatotoxicity treatment [10]. *Cassia occidentalis* is a pantropical plant species and it is popularly known as coffee senna, stinking weed, miki palaoa, hedionda, bucho, pico de pájaro, furrusca, bois puante, fedegoso, rai dore and many other

names. It belongs to the family leguminosae, sub-family caesalpinoideae and genus *cassia*. It is botanically classified as both *Cassia occidentalis* and *Sennaoccidentalis*[11]. It is a small, erect, annual herb that can be up to 2m tall and is commonly found by road sides' ditches and waste dumping sites of sub-Saharan African countries. Its seeds are found in long seed pods, and are sometimes roasted and made into coffee like beverage. *Cassia occidentalis* has a rich history in natural medicine and the parts of the plant used include roots, leaves, and seeds as well as the whole plant in some occasions [12].

2. Materials and Methods

2.1 Preparation of plant extract (methanolic extract)

The plant material was obtained from within Kaduna metropolis, Kaduna State, Nigeria, in the month of March 2015 and was identified and authenticated in the Department of Biological sciences, Ahmadu Bello University, Zaria. Preparation of whole plant extract was done by soaking 150g of fine powdered in 700ml 85% methanol for 48 hours after shade drying, then filtered using whatman filter paper No. 1 and concentrated in rotary evaporator to obtain a dark green semi solid sample, according to [13] Azmi and Qureshi (2012), and it was kept at 0°C until needed.

2.2 Phytochemical analysis

The MECO whole plant was screened for the presence saponins, tannins, anthraquinones, cardenolides, flavonoids, glycosides, terpenoids, steroids and alkaloids according to the methods described by [14].

2.3 Chemicals Used

Normal saline (n-Saline) and Simvastatin (20 mg) were purchased from Alfa chemical company ltd, Kaduna-Nigeria. Lipid profile diagnostic reagent was procured from Agappe chemical company, Switzerland and Triton X-100 was obtained from Dr T Bulus, Department of Biochemistry, Kaduna State University, Kaduna-Nigeria. All chemicals and reagents used are of analytical grade.

2.4 Experimental Animals and Triton-X100 induction

Thirty albino rats weighed 150-210 g were purchased from the animal house of Nigeria Institute for Trypanosomiasis and Onchocerciasis Research (NITOR) Kaduna State. The rats were acclimatized in conventional animal house according to the internationally accepted guidelines for animal handling. They were allowed free access to both standard laboratory diet and water *ad libitum* throughout the period of the experiment. Hyperlipidaemia was induced by single subcutaneous administration of Triton X-100 (150 mg/Kg body weight). The rats with elevated level of serum lipid profile parameters three days post-induction, were considered hyperlipidemic and used for this research work.

2.5 Determination of acute toxicity of MECO

Acute toxicity of MECO was evaluated by administering (250–4000 mg/kg body weight) dose of same extract orally

in three rats of a group of five overnight fasted albino rats with the remaining two serving as control, treated with normal saline in a dose of 1 ml/kg body weight. After receiving treatment, the rats were kept in observation for the period of 24 hours to monitor behavioural changes and signs like tediousness, sedation, ruffled hair, clumping together, itching, restlessness and mortality based on dosage as described by [15].

2.6 Determining Antihyperlipidemic Activity of MECO

Twenty five rats were divided into five groups of five rats each and were treated as follows:

Group 1: Normal rats were given 1ml of normal saline

Group 2: Hyperlipidemic control rats were given 1ml of normal saline

Group 3: Hyperlipidemic rats were given MECO (200 mg/kgb.w) once daily

Group 4: Hyperlipidemic rats were given MECO (400 mg/kgb.w) once daily

Group 5: Hyperlipidemic rats were given Simvastatin (5 mg/kgb.w) once daily

After the treatment with the extract for 21 days, the rats from each group were fasted overnight and sacrificed. Blood samples were collected in a centrifuge tubes and separated for the estimation of total lipids (i.e. total cholesterol, triglyceride, LDL, HDL and vLDL).

2.7 Biochemical Analysis

Serum cholesterol was estimated according to the method described by [16], serum triglycerides were estimated according to the method described by [17]. HDL-Cholesterol, LDL-Cholesterol were also estimated according to the method described by [18] and vLDL-Cholesterol was determined according to the expression given by [19].

2.8 Statistical Analysis

One-way Analysis of Variance (ANOVA) was carried out using SPSS statistical software version 21 by IBM Inc. Followed by Student-Newman-Keuls Multiple Comparisons Test. The data were expressed as mean ± Standard deviation. The p-values ($p < 0.05$) were considered significant.

3. Result

Basically, the result of qualitative phytochemical analysis of MECO revealed the presence of saponins, tannins, anthraquinones, cardenolides, flavonoids and alkaloids, detail in Table 1.

Table 1: Summary of phytochemical constituent of MECO

Constituents	indication
Alkaloids	+ve
Anthroquinones	+ve
Cardenolides	+ve
Flavonoids	+ve
Glycosides	-ve
Saponins	+ve
Steroids	-ve
Tannins	+ve
Terpenoids	-ve

+ve stands for presence while -ve stands for absence

The acute toxicity of MECO was evaluated in two stages in which the rats were administered 2000 mg/kg.b.w of the extract orally in the first stage and no mortality was recorded. The dose was multiplied by two making it 4000mg/kg.b.w and no consequent mortality was recorded upon administered. After fourteen days, the rats were observed and their weights were measured and compared with their initial weights. No behavioural changes were recorded in the rats under investigation and the weight difference was not statistically significant ($p > 0.05$). Thus, MECO extract could be considered practically non-toxic since the LD_{50} is greater than 4000 mg/kg.b.w.

Table 2: Serum lipid profile in Triton X-100 induced hyperlipidemic rats after 21 days of oral administration of Methanolic Extract of *Cassia occidentalis* whole plant and Simvastatin.

Groups	T-dose (mg/kg b.w)	Serum parameters (mg/dL)				
		CHOL	TGR	HDL	LDL	VLDL
NC	n-saline	57.08±5.34	193.95±13.35	34.42±0.75	26.14±4.48	38.79±2.67
HLDC	n-saline	220.75±7.14*	423.35±8.78*	21.41±0.81*	79.33±2.11*	84.67±1.78*
MECO	200	177.07±8.07 ^{ba}	331.96±19.79 ^{bb}	31.06±0.72 ^{ba}	47.38±3.73 ^{bb}	66.39±5.36 ^{bb}
MECO	400	162.23±8.41 ^{ca}	312.33±18.21 ^{cc}	35.11±0.51 ^{ca}	31.02±3.52 ^{cc}	58.22±4.33 ^{cc}
SVTN	05	158.56±8.95 ^{da}	137.14±15.05 ^{da}	34.92±1.10 ^{da}	22.77±4.87 ^{da}	27.25±2.83 ^{da}

Results are expressed as mean ± SD. Values with asterisk symbol in each column differed significantly ($p < 0.050$) with normal control (NC). Values bearing superscripts in each column are also significantly different at $p < 0.050$ compared to hyperlipidemic control (HLDC). MECO: Methanolic extract of *cassia occidentalis*, SVTN: Simvastatin (standard drug).

Table 2 above shows the effect of MECO on the serum level of cholesterol, triglyceride, HDL, LDL and VLDL at different doses after 21 days of treatment. All the parameters were found to be significantly ($p < 0.050$) higher in the hyperlipidemic rats when compared to normal control rats except the HDL which was significantly lower in the comparison. Significant decrease in the serum levels of cholesterol, triglyceride, LDL and VLDL, and elevated level of HDL was recorded in the two groups that received different doses of the MECO orally when compared with values in hyperlipidemic rats. The serum level of triglyceride, LDL and VLDL in simvastatin treated rats was found to be significantly ($p < 0.050$) lower in comparison to the two groups treated with different doses of the extract. In turn, the serum level of cholesterol and HDL in simvastatin treated rats were slightly different ($p > 0.050$) when compared with the values in the two groups treated with MECO at different doses.

4. Discussion

Hyperlipidaemia is a major risk factor in the pathogenesis of atherosclerosis, a physiologic disorder that affects the coronary, cerebral and peripheral arterial circulation leading to ischemia [20]. It is one of the many metabolic abnormalities regarded as world leading killer disorders such as Diabetes, Coronary heart diseases, Obesity to mention a few. Abnormal level of plasma lipid parameters resulting from unhealthy food, sedentary life style and deficiency in counter-regulatory hormone lead to the various metabolic abnormalities observed in hyperlipidemia which causes damage, dysfunction and failure of various organs [21]. Many medicinal plants have been the substitute of orthodox medicine in many countries to which Nigeria is not left out. Despite their widespread use, few studies have reported to ascertain the efficacy of their traditional remedies [22].

According to the WHO's expert committee recommendation on metabolic diseases (1987), it is essential to investigate and ascertain hypolipidemic effect of plants that are traditionally used in place of orthodox medicine. *Cassia occidentalis* (*C. occidentalis*) is found mostly in tropical areas worldwide, where it is used as medicinal plant in varying capacity for decades [23]. The plant exhibits the presence of many bioactive compounds including flavonoids, alkaloids, glycosides steroids terpenoids, polysaccharides and peptidoglycans [24] that have various effects on several metabolic and inflammatory diseases.

In this study, phytochemical screening of MECO revealed the presence of many active compounds as seen in table 1 above. The presence of these various active compounds may be responsible for the physiology and or biological activity of the plant. Among the phytochemicals listed, tannins was reported to reduce the risk of coronary heart disease which in a way reduce the chance of plaque formation by circulating fats [25]. Thus, may be responsible for ameliorative effect in this study. Subcutaneous (SC) administration of Triton-X-100 induced hyperlipidemia after 48 hours. Evidently, Triton-X-100 has been widely reported to induce hyperlipidemia by blocking the clearance of triglycerides-rich lipoprotein to induce acute hyperlipidemia in various laboratory units such as albino rats [26].

Oral administration of both 200 and 400 mg/kg body weight of MECO led to significant reduction ($p < 0.05$) in plasma cholesterol, triglycerides, LDL, and VLDL except HDL which had significant increase ($p < 0.05$) in hyperlipidemic rats when compared with the hyperlipidemic control after the designated 21 days (period of treatment) (Table 2). Similar effects have been recorded in other studies using the leaves of *Cassia occidentalis* alone [27], the flower only [28]. Many studies have found Simvastatin to significantly

lower the level of plasma lipids parameters which is in agreement with the observation made in this present study.

The nature of changes observed in the groups treated with standard drug and that of 400 mg/kg body weight is more pronounced than those treated with 200 mg/kg body weight, that is to say this significant changes in the lipid parameters were dose dependant. Thus, this result was in agreement with the previous reports in literature [29], [30] and [31]. The observation could be attributed to the fact that majority of drugs/plants extracts' bioavailability reflect their concentrations, i. e the higher the dose the higher the bioavailability as well as the efficacy. Probably, if the dose is not enough, the active principles either form complexes with polyvalent ions, undergo hydrolysis by gastric acid or digestive enzymes and conjugation in the intestine, thus affecting the clearance of insufficient dose of drug absorbed into circulation [32].

Simvastatin is among the group of antihyperlipidemic drugs considered effective in reducing the level of deterioration in the body caused by abnormal plasma lipids by inhibiting HMG-reductase and thereby lowering the lipid level [33]. Study has shown that most plants exert their effect on metabolic syndromes by interfering with the process of absorbing monomeric units of the macromolecules directly involved in the development of such disease condition [34]. More so, it was reported that Saponins and Tannins in plants exhibit antihyperlipidemic activity, the former was believed to enhance the synthesis of transport proteins (lipoproteins) for faster blood lipid clearing effect. The presence of active principles in the plants is believed to be sole responsible for this activity, and the later was reported to potentially inhibit the activity of lipase found in rats which could perhaps be reflected in human lipase [34], thus, prevent lipid deposition in the vessels [36]. Interestingly, Saponins and Tannins were highly present in methanolic whole plant extract of *C. occidentalis* in this study, and it may be the explanation for the antihyperlipidemic activity observed in the study.

Likewise, previous studies carried out on plants (*Cassia fistulata* and *Cassia alata*) of the same family with *Cassia occidentalis* exhibited similar findings with the present studies which was also attributed to the high concentration of tannins present in the plants [37],[38]. Hyperlipidemia/Dyslipidemia is associated with Type two diabetes mellitus (T2DM) which mark the alterations in the level of serum insulin or resistance to insulin by their respective receptor molecules [39]. This abnormal behaviour of insulin in hyperlipidemia is mainly due to improper lipids metabolism since there is opposite action between Insulin and hormone sensitive lipase [40].

The serum lipid lowering effect of MECO whole plant observed in the present study may have strong association with inhibition of endogenous synthesis of lipids by perhaps enhancing the insulin secretion, inhibiting hormone sensitive lipase and or decreasing the mobilization of free fatty acids from adipose tissues. However, phytochemicals such as flavonoids in plant extracts are reported to contribute immensely to the serum lipid lowering effect in rats due to their high antioxidant activity [41] and flavonoid level is observed to be high in MECO used in the present study.

5. Conclusion

The results obtained in the present study signified that methanolic extract of *Cassia occidentalis* (MECO) whole plant can be a good source of potent antihyperlipidemic agent capable of ameliorating the metabolic abnormalities associated with hyperlipidemia which include coronary heart diseases, diabetes mellitus especially type two, and obesity.

6. Future Study

Future study will focus on the structural elucidation and molecular mechanism of action of the purely isolated form of the active principles (Saponine, Tannine and Flavonoids) responsible for the antihyperlipidemic effect of MECO using appropriate cell line(s).

7. Acknowledgement

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