



Antidiabetic activity of the methanol and acetone extracts of twigs of *Combretum molle* in dexamethasone induced-insulin resistance in rats

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ABSTRACT

Combretum molle plant has applications in African traditional medicine against pain, diabetes mellitus and microbial diseases. In present study, we investigated the antidiabetic potential of the methanol and acetone extracts of twigs of *C. molle* in dexamethasone induced-insulin resistance diabetes mellitus. Single of extracts (250 and 500 mg/kg, *p.o.*) was administered to normal and hyperglycemic animals. Insulin resistance was induced by daily sub-cutaneous injection of dexamethasone at dose of 1 mL/kg. The anti-diabetic effects were also evaluated, by measuring glycaemia, body weight, serum levels of lipid parameters and atherogenic indices and coronary artery risk. These studies revealed that oral administration of both extracts (methanol and acetone) to normal and hyperglycemic rats significantly suppressed the rise in blood glucose level, as glibenclamide. Oral treatment with dexamethasone injection for 10 days was associated with significant weight loss, hyperglycemia, hyperlipidemia and atherogenic indices. However, pretreatment with extracts significantly prevented increase in any of these measured parameters. Results of this study suggest that the hypoglycemic, anti-hyperlipidemic and anti-atherogenic effects of these extracts are mediated through increased peripheral glucose uptake and improvements in insulin resistance, thus, validating its ethnomedical use in the traditional management of diabetes mellitus.

Keywords: Dexamethasone, insulin resistance, antidiabetic activity, *Combretum molle* twigs, methanol and acetone extracts.

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic diseases characterized by chronic hyperglycemia or increased blood glucose levels with disturbances in carbohydrates, protein and fat metabolism resulting from insulin deficiency, impaired effectiveness of insulin's action, or to a combination of both [1]. The disease has reached epidemic proportions in the current century. DM is a common health problem worldwide, and the prevalence and incidence of this disease are rapidly increasing [2]. The world's prevalence of diabetes among adults (aged 20-79 years) was 6.4%, affecting 285 million adults in 2010 and will increase to 7.7% *ie.* 439 million adults by 2030 [3]. India leads the world today with the largest number of diabetics with a mean annual increment of 1.8 million [3]. Of all

these diabetic populations, about 90% account of type 2 diabetes [4].

Clinical studies on different species of animals have shown that consuming less food reduces the risk of diabetes and heart disease. Current treatment of type 2 diabetes remains inadequate; prevention and leaving are preferable weapon [4]. One therapeutic approach for treating DM is decrease postprandial hyperglycemia. Several antihyperglycemic drugs such as biguanides, sulfonylureas, thiazolidinediones and alphasglucosidase inhibitors are the cornerstone of diabetes treatment; they have important adverse effects and cannot always maintain euglycemia and prevent diabetes complications significantly [5]. If diabetes is not duly treated, it will lead to long term damage, dysfunction and failure of various organs

and systems, especially the eyes, kidney, nerves, limbs and cardiovascular system [6]. The complications of diabetes are the main causes of morbidities and mortalities [7,8]. However, all these treatments have limited efficacy and have been reported to be associated with undesirable side effects [9].

In order to overcome the side effects associated with diabetes, interest has been shifted to use of other alternative medicine. Traditional medicines and extracts from medicinal plants have been extensively used as alternative medicine for better control and management of diabetes mellitus. Medicinal plants have continued to be a powerful source for new drugs, now contributing about 90% of the newly discovered pharmaceuticals [10]. In fact, many developing countries around the world use traditional medicine, because it is sometimes the only affordable source for healthcare. As for the developed countries, the use of herbal medicine for chronic diseases is encouraged because there is concern about the adverse effects of chemical drugs and treatment using medicines of natural origin appears to offer more gentle means of managing such diseases. Herbal drugs are prescribed widely because of their effectiveness, fewer side effects and are relatively low in cost. Plants have long been used for the treatment of diabetes, particularly in developing countries where most people have limited resources and do not have access to modern treatment. Ethnobotanical studies have reported more than 1200 plant species with potential antidiabetic effects [11]. *Combretum molle* (Combretaceae) is a shrub or small, graceful, deciduous tree 3-13 m high; trunk crooked or leaning, occasionally swollen at the base, up to 30 cm in diameter. It is found especially in savannah vegetation that cuts across from Senegal to West Cameroon, but generally exists in tropical Africa [12]. *C. molle* is widely used in African traditional medicine for the treatment of various diseases. It is reported to possess antifungal, antimicrobial, antiparasitic, antioxidant, and anti-inflammatory and antidiabetic pharmacological properties [13]. Therefore there is a depth of information regarding the bioactivity of the different parts of this plant which merits further investigation.

This study was therefore undertaken to find out whether in the case of *C. molle* twigs acetone and ethanol extracts could be effective in the management of diabetes in dexamethasone-induced insulin resistance in rats.

MATERIALS AND METHODS

Chemicals and drugs: Alloxan, acarbose, and kits for biochemical dosages were purchased from

Sigma-Aldrich, St. Louis, USA. Starch, D-glucose and sucrose were purchased from Edu-Lab Biology Kit, Bexwell, Norfolk PE38 9GA, UK. All chemicals and drugs were obtained commercially and were of analytical grade. For dilutions, metformine, glibenclamide, starch, acarbose, glucose and sucrose were dissolved in normal saline. The extracts were dissolved in physiological saline by DMSO (dimethyl sulfoxide). All drugs were prepared immediately before use.

Experimental animals: Nulliparous and non-pregnant healthy albinos female rats aged 8-12 weeks, weighing between 160-180 g were used for toxicological test and albinos males rats aged 3-4 months, average weight 250 g were used for pharmacological tests. These animals were raised in the animal house of the Department of Animal Biology, Faculty of Sciences at the University of Dschang, Cameroon, were used. The animals were housed in plastic cages and maintained in ambient temperature of $24 \pm 1^{\circ}\text{C}$, relative humidity of 55-65% and normal light/dark cycle. They were fed standard laboratory diet and water *ad libitum*. The rats were acclimatized to laboratory condition for one week before commencement of experiments. The Ethical clearance for the usage of rats was obtained from the Institutional Animal Ethical Committee (IAEC) prior to the beginning of the study [14].

Plant material: The fresh twigs of *Combretum molle* were collected in the month of December from village Moutourwa, situated near Maroua city (Far North Region, Cameroon). The plant material was authenticated in the National Herbarium, Yaoundé where voucher specimen was deposited. After authentication, the collected fresh twigs were cleansed thoroughly under running tap water, dried under shade and ground into fine powder.

Preparation of extracts: The dried powder of *C. molle* (200 g) was soaked in 1000 mL of each solvent (methanol and acetone) for 72 h in cold condition. The whole mixture was successively filtered through a piece of clean, white cotton material and No.1 Whatman filter paper. The filtrate obtained was evaporated at 80°C using rotary evaporator to obtain 19.35 g and 14.12 g representing a yield of 9.67 % and 7.48% for methanol and acetone extracts respectively.

Qualitative phytochemical tests: The extract obtained was subjected to the preliminary phytochemical analysis, which included tests for alkaloids (Dragendoff reagent), saponins (frothing test), tannins (FeCl_3), glycosides (Legal test), flavonoids (NaCl and HCl), reducing sugars (Fehling liquor), steroids (chloroforme and H_2SO_4

concentrate), terpenoids (chloroforme and H₂SO₄ concentrate) and phenols (FeCl₃ and K₃Fe (CN)₆). These were identified by characteristic color changes using standard procedures [15].

Acute toxicity: Acute toxicity studies were performed according to the Organization for Economic Co-operation and Development (OECD) guidelines 425 [16]. Ten animals were divided in two groups of 5 animals each. The rats were fasted for 6 h with free access to water only. The acetone extract and DMSO were administered orally in doses of 2000 mg/kg and 10 mL/kg to different groups of female rats. Observations were made and recorded systematically 30 min, 4 h, 24 h, 48 h, 7 days and 14 days after dose administration for skin and fur changes, eyes, salivation, lethargy, sleep, coma, convulsion, tremors, diarrhea, mortality, mobility, aggressivity, sensitivity of the sound, touch and pain as well as respiratory movement.

Normoglycemic study: Overnight fasted rats were divided 6 groups consisting of 6 animals of each group. Group I rats received normal saline (10 mL/kg, *p.o.*) as vehicle. Group II rats received glibenclamide (0.3 mg/kg, *p.o.*) as standard drug. Groups III and IV rats received methanol extract of *C. molle* (MECM) at the doses of 250 and 500 mg/kg body weight, *p.o.* in a single dose. Groups V and VI rats received acetone extract of *C. molle* (AECM) at the doses of 250 and 500 mg/kg body weight, *p.o.* in a single dose. The tail was snipped and blood glucose level of each rat was tested using the glucometer (One touch^R ultra 2, Blood glucose meter, Life Scan Europe, 6300zug, Switzerland). Blood sample was tested just before oral administration of substances (0 hour) and at 2 and 4 hours in each case.

Oral carbohydrate challenge tests in normal rat [17]: The oral carbohydrate tolerance tests were carried out using glucose, starch and sucrose separately in normal groups of rats and were equally divided into various treatment groups. In oral glucose tolerance test, rats were divided into 6 groups consisting of 6 rats in each group. The rats were fasted overnight for 16 hours but had free access to water. Group I rats were treated orally with normal DMSO 5% 10 mL/kg (normal control). Group 2 rats were treated orally with glibenclamide 10 mg/kg body weight (positive control). Groups III and IV animals received 250 and 500 mg/kg MECM. Groups V and VI rats received 250 and 500 mg/kg AEMC. The rats in all the groups were administrated with D-glucose (3 mg/kg, *p.o.*), 60 min after extract administration. Blood samples were collected from the tail at 30, 60, 90 and 120 min after glucose administration. Blood glucose levels were measured immediately

with a glucometer one touch. The oral starch and sucrose tolerance tests were carried out with DMSO 5%, acarbose, MECM and AECM in the same way as above, but in these tests, starch and sucrose at a dose of 4 g/kg body weight each was used.

Dexamethasone induced insulin resistance: The study was carried out for 11 days to access the effect of various treatments on biochemical parameters of different tissues in dexamethasone induced insulin resistance in rats. The rats were divided into 7 groups, consisting of 5 animals each. Animals were kept for overnight fasting 16 hours prior to experiment. Rats in the first group received normal saline (10 mL/kg, *p.o.* + 1 mL/kg, *s.c.*) and served as normal control. The second group of rats received normal saline (10 mL/kg, *p.o.*) and served as diabetic control group (negative control group). Rats in the third group were treated with metformin (40 mg/kg, *p.o.*) and served as positive control group. Rats in experimental group 4 and 5 were treated with MECM (250 and 500 mg/kg *p.o.* respectively). Rats in the group 6 and 7 received AECM (250 and 500 mg/kg *p.o.* respectively).

One hour after pretreatment, all rats in the treatment groups II-VII were subcutaneously ingested with 1 mL/kg of dexamethasone sodium phosphate. The animals were weighed every day during all the treatment. Glycemia of each overnight fasted animal was estimated as above but, this time at the beginning and at the end of the experimental period.

Oral glucose tolerance in diabetic rat: After an overnight fast on the 11 days of the experiment, the fasting blood glucose levels of rats in all the treated groups were determined using one touch ultra-glucometer. 90 min afterwards, oral tolerance test was conducted in all the drug treated rats with 3 g/kg of D-glucose. The blood glucose levels were monitored at 30, 60, 90 and 120 min after administration of glucose. Following the oral glucose tolerance test, the animals were deprived of food overnight (12 h), anesthetized with diazepam/ketamine, *i.p.* and sacrificed. Whole blood samples were taken from the abdominal artery by catheterization method. The blood samples left for 15 minutes at 37°C for serum separation, then centrifugated at 3000 rpm for 20 minutes, then sera were separated and kept in eppendorf tubes at -20°C until analyses. Just after the blood collection samples, organs such as kidneys, heart, pancreas, liver, spleen and lungs were removed and weighed.

Biochemical Analysis: Total cholesterol (TC) was determined in serum according to the enzymatic

colorimetric method described by [18] using DIALAB kit. Triglycerides (TG) were determined in serum according to the enzymatic colorimetric method described by [19] using INMESCO kit. Serum high density lipoprotein cholesterol (HDL-c) concentration was determined in serum according to the enzymatic colorimetric method described by [20] using INMESCO kit. Serum low density lipoprotein cholesterol (LDL-c) concentration was calculated by reduction of the sum HDL-c and LDL-c concentrations from that of TC, using equation: $LDL-c = TC - (TG/5) - HDL-c$.

Determination of atherogenic and coronary artery risk indices: Atherogenic index (AI) was calculated according to the methods of [21] and expressed as $AI = (TC-HDL-c)/HDL-c$. Coronary artery risk index (CRI) was also calculated using the formula $TC (mg/dl)/HDL-c (mg/dl)$ [22].

Statistical analysis: Statistical analysis was performed using the statistical functions of the Graph pad Prism version 4.1. All the results were expressed as mean \pm SEM. The significance of difference between mean values for the various treatments were tested using one-way analysis of variance (ANOVA) followed by Turkey test and two-way analysis of variance followed by Bonferroni test. Statistical significance was considered at $p < 0.05$; $p < 0.01$ and $p < 0.001$.

RESULTS

Preliminary phytochemical screening: The preliminary phytochemical screening revealed the presence of glycosides, saponins, flavonoids, phenols, triterpenoids and tannins.

Acute oral toxicity study: Acute oral administration of a dose 2000 mg/kg of acetone extract of *C. molle* twigs produced no mortality after 24 hours and after 14 days of observation period. The extract treated female rats did not produce any grossly negative physical and behavioral changes. The LD50 value was found to be more than 2000 mg/kg body weight. Hence, about one tenth of this dose (250 mg/kg) was selected as the level for examination of antidiabetic potential.

Normoglycemic study: The normoglycemic study showed that all rats treated with glibenclamide (0.3 mg/kg) produced a significant ($P < 0.01$ and $P < 0.05$) reduction in blood glucose level as compared to control group, 2 and 4 hours after administration. Similar results were obtained with AEMC at two doses 250 and 500 mg/kg. However, MECM at a dose of 250 mg/kg orally did not produced any

blood sugar level while a dose of 500 mg/kg of the same extract significantly ($p < 0.05$) decreased the blood glucose level after 4 hours, as compared with control group (Table 1).

Oral glucose tolerance test in normal rat: The administration of glibenclamide and graded doses of MECM and AECM orally showed improved glucose tolerance in normal rats (Figure 1). In fact, in glibenclamide group rats, oral glucose administration was associated with a significant and time-dependant reduction in blood glucose level at 60 ($P < 0.05$), 90 ($P < 0.001$) and 120 min ($P < 0.001$) when compared to vehicle control animals. Similar pattern of postprandial glucose changes was recorded for rats treated with AECM at dose 500 mg/kg while in the case of AEMC a dose of 250 mg/kg, reduction effect was observed 90-120 min after glucose load ($P < 0.05$). However, MECM at a dose of 250 mg/kg induced a significant ($P < 0.05$) and transitory decrease in blood glucose level at 90 min while the higher dose of the same extract reduced blood glucose level significantly at 90 ($P < 0.01$) and 120 min ($P < 0.05$), in comparison with control group rats.

Oral starch tolerance test in normal rat: In normal rats, MECM at two doses and AECM at dose 250 mg/kg did not show any significant decrease in blood glucose level compared to control rats (Figure 2). However, AECM at dose 500 mg/kg, reduced blood glucose level significantly ($P < 0.05$) at 90 min. Acarbose significantly reduced the blood glucose level at 60 ($P < 0.05$), 90 ($P < 0.01$) and 120 min ($P < 0.01$).

Oral sucrose tolerance test in normal rat: In normoglycemic rats, none of the MECM-treated groups showed any reduction in blood glucose level as compared to normal control group (Figure 3). However, AECM at dose 250 mg/kg showed a significant ($P < 0.05$, $P < 0.01$ and $P < 0.05$) decrease in blood glucose at 60 min, 90 min and 120 min respectively. At 60 min, 90 min and 120 min, AECM at dose 500 mg/kg reduced blood glucose level significantly ($P < 0.01$). Both AECM at dose 500 mg/kg and acarbose failed to significantly ($P < 0.05$) suppress the lower blood glucose level at 60 min, 90 min and 120 min; but at 30 min after sucrose administration, acarbose produce significant decrease ($P < 0.05$) in blood glucose level compared to control group rats.

Effect of *C. molle* extracts on blood glucose level in dexamethasone-induced insulin resistance in rat: The effect of methanol and acetone extracts on blood glucose level of dexamethasone induced insulin resistance in rats is presented in Figure 4. Results showed that the administration of

dexamethasone (10 mL/kg) resulted in insulin resistance as evidenced by the significant ($P < 0.001$) increase in mean glucose (45.60 ± 2.63 mg/dL) of the diabetic control when compared with the normal control. Continuous treatment with metformine and extracts of rats of the respective groups for 10 days along with dexamethasone showed significant decrease in blood glucose level when compared to diabetic control group. Metformine, MECM at dose of 500 mg/kg and AECM at doses of 250 and 500 mg/kg along with dexamethasone produced a significant ($P < 0.001$) decrease in mean glucose (38.4 ± 1.19 mg/dL, 34.20 ± 0.07 mg/dL, 35.20 ± 1.63 mg/dL and 39.80 ± 1.98 mg/dL) respectively, when compared to diabetic control group. However, all animals treated with MECM at dose of 250 mg/kg along with dexamethasone reduced glycaemia by about 14.80 ± 1.81 mg/dL significantly ($P < 0.01$) as compared to diabetic control group.

Oral glucose tolerance test in diabetic rat: As shown in Figure 5, the diabetic control group rats showed a significant increase in post-prandial glucose concentration at 60 ($P < 0.01$), 90 ($P < 0.001$) and 120 min ($P < 0.001$) in comparison to normal control group, whereas dexamethasone + MECM 250 mg/kg treated group showed tendency in postprandial blood level reduction at 90 min ($P < 0.05$). On the other hand, metformine and AECM at dose of 500 mg/kg along with dexamethasone demonstrated a significant ($P < 0.01$, $P < 0.01$ and $P < 0.01$) drop in blood glucose level at 60, 90 and 120 min when compared to diabetic control, while the lower dose of AECM along with dexamethasone shifted and delayed the blood glucose concentration from 90 ($P < 0.01$) to 120 min ($P < 0.01$). Furthermore, diabetic rats treated with a combination of dexamethasone and MECM at dose of 250 mg/kg, showed a significant ($P < 0.01$) decrease in blood glucose level at the end of 120th min as compared to diabetic non-treated animals. However, at 60, 90 and 120 min, the higher dose of MECM decreased blood glucose level significantly ($P < 0.05$; $p < 0.001$; $P < 0.001$) in experimental rats respectively, when compared to dexamethasone group.

Effect of *C. molle* extracts on body and organs weights in dexamethasone-induced diabetic rats: Table 2 show the effect of the 10 days of oral treatment with the methanol and acetone extracts on the body weight and organs weight of treated rats. The results showed that, metformine and AECM at dose of 500 mg/kg produced about 29% and 25% increase in body weight ($P < 0.01$) at the end of 10th day of their treatment respectively in comparison to diabetic control. However, the body weights of rats treated as above were reverted to

near normal when compared to normal control. There was a 15.40 % reduction in body weight ($P < 0.001$) in dexamethasone treated rats when compared to normal control. The same results were observed with 250 mg/kg AECM and both MECM at doses 250 and 500 mg/kg; this reducing and dose-dependent effect was about 10.09 % ($p < 0.05$), 11.80 % ($p < 0.01$) and 9.22 % ($p < 0.05$) respectively.

Furthermore, administration of metformine, dexamethasone and different extracts of *C. molle* prior to dexamethasone injection resulted in a non-significant difference between the weight of kidneys, heart, lungs and pancreas of this group and the normal control group. However, negative control rats produced a significant ($p < 0.001$ and $p < 0.01$) increase in the weight of liver and spleen to about 37.01 % and 50 % respectively. In comparison to diabetic control, all rats pretreated with metformine, MECM at doses of 250 and 500 mg/kg and with AECM at doses of 250 and 500 mg/kg demonstrated a significant and dose-dependent drop in liver weight of about 16.22 % ($p < 0.01$), 18.92 % ($p < 0.01$), 32.43 % ($p < 0.001$), 24.32 % ($p < 0.001$) and 24.32 % ($p < 0.001$) respectively. Spleen weight of animals pretreated with both AECM at doses of 250 and 500 mg/kg was also significantly ($p < 0.01$) lower (about 56.25 % and 31.25 % respectively) than those of the diabetic group, whereas MECM at doses of 250 and 250 mg/kg did not produce any significant variation in spleen weight.

Effect of *C. molle* on serum lipids and cardiovascular risk indices in dexamethasone-induced insulin resistance in rats: In Table 3, the lipid parameters, AI and CRI of extract treated groups were compared with the diabetic control group. Results indicate that, in comparison to normal control, dexamethasone group produced a significant ($p < 0.001$) mean difference in the TC (17.98 ± 1.34 mg/dL), TG (20.09 ± 0.47 mg/dL), HDL-c (18.23 ± 0.84 mg/dL), LDL-c (32.19 ± 0.43 mg/dL), AI (1.70 ± 0.24 mg/dL) and CRI (1.70 ± 0.24 mg/dL) values. Animals pretreated with metformine for 10 days at a dose of 40 mg/kg showed a significant ($P < 0.001$) decrease in serum TC (26.08 ± 0.53 mg/dL), TG (25.74 ± 3.20 mg/dL), LDL-c (39.91 ± 0.56 mg/dL), AI (1.90 ± 0.25 mg/dL) and CRI (1.90 ± 0.25 mg/dL) levels, and a significant ($p < 0.001$) increase in serum HDL-c (18.97 ± 1.11 mg/dL), comparatively to diabetic control. Similar effect was also observed on the serum TC, TG, HDL-c, LDL-c, AI and CRI of pretreated rats groups with both extracts.

DISCUSSION

Insulin resistance and hyperinsulinemia are often associated with a group of risk factors as obesity, atherosclerosis, dyslipidemia, hypertension and impaired glucose tolerance. Any interventions therefore to decrease insulin resistance may postpone the development of diabetes and its complications. Treatment with herbs has been a better choice because they are effective with fewer side effects and are affordable as compared to presently used synthetic oral antidiabetic drugs. The present study was undertaken to investigate the antidiabetic effects of *Combretum molle* twigs extracts in dexamethasone induced diabetic rats as an experimental model of type 2 diabetes mellitus.

Acute toxicity test at 2000 mg/kg body weight of the AECM produced no mortality after 14 days of observation which indicates that the mean lethal dose (LD50) of the extract is greater than 2000 mg/kg body weight. Generally, acute toxicity did not produce any grossly negative behavioral changes in the rats it instead reduced reaction to noise was observed suggesting that, the extract may have depressant effect on the central nervous system [23].

The ability of MECM and AECM to effectively control increased blood glucose level in normal and diabetic rats may be attributed to its hypoglycemic and antihyperglycemic effects. In fact, normoglycemic study revealed that the high dose (500 mg/kg) of MECM and both doses (250 and 500 mg/kg) of AECM reduced blood glucose level significantly ($p < 0.05$) 2-4 hours post treatment. Oral administration of both extracts to rats fed with glucose significantly ($p < 0.05$; $p < 0.01$ and $p < 0.01$) suppressed the rise in blood glucose level in both normal and diabetic rats, as glibenclamide or metformine. These results suggest that animals treated with extracts have better glucose utilization capacity suggesting its mechanism being almost similar to reference products (glibenclamide or metformine). They promote tissue glucose uptake and reduce hepatic glucose output, thereby producing hypoglycemic and antihyperglycemic effects. However, both doses of AECM only were administered to sucrose loaded normal fasted rats resulting in hypoglycemia ($p < 0.05$; $p < 0.01$ and $p < 0.01$), whereas MECM did not affect the rise in glycaemia. Moreover, concerning oral starch tolerance test, AECM at dose 500 mg/kg only reduced blood glucose level significantly ($P < 0.05$) at 90 min. The above results show a striking similarity to the effects of acarbose. These findings suggested that AECM could decrease the postprandial glucose level by inhibiting the activity of α -amylase (weakly) and α -glucosidase, which

are important enzymes in the digestion of the complex carbohydrates into absorbable monosaccharides in the food [24]. Recently, some reports showed that glycosides and triterpenoids could effectively inhibit the activity of α -amylase and/or α -glucosidase to decrease the absorption of carbohydrates from food [25,26]. So the glycosides and triterpenoids in AECM might take the responsibility for the postprandial antihyperglycemic effect of extract. It was very interesting to point out that AECM was more efficient for complex carbohydrate sucrose than the starch. Acarbose like drug that inhibit α -glucosidase present in the epithelium of the small intestine, have been demonstrated to decrease postprandial hyperglycemia and improve impaired glucose metabolism without promoting insulin secretion in type 2 DM patients [27]. These medications are most useful for people who have just been diagnosed with type 2 diabetes and who have blood glucose levels only slightly above the level considered serious for diabetes.

The present study indicated that daily administration of *C. molle* (250 and 500 mg/kg) up to 10 days showed anti-diabetic and hypolipidemic effects in diabetic rats. In fact, high exposure to glucocorticoids impairs insulin sensitivity, contributing to the generation of metabolic syndrome including insulin resistance, hyperglycemia and dyslipidemia in human and experimental animals [28]. The mechanism by which dexamethasone induces peripheral insulin resistance is by inhibiting GLUT-4 (glucose transporters) translocation from intracellular compartments to the plasma membrane particularly of skeletal muscles. The extracts could act by reversing the glucocorticoid mediated translocation of the glucose transporters from the plasma membrane to the intracellular compartment. The twigs extracts of *C. molle*, therefore, appeared to have improved insulin resistance through enhanced insulin sensitivity in peripheral tissues, as was evident from the decreased glucose levels. Moreover, dexamethasone action is mediated by the glucocorticoid receptor, a nuclear receptor that regulates physiological events through activation or repression of target genes involved in inflammation, gluconeogenesis and adipocyte differentiation [29]. It is therefore, probable that the *C. molle* extracts may contain substances that compete with dexamethasone for the glucocorticoid receptor (competitive antagonism) or they may contain substances that stimulate the production of repressor elements that inhibit the transcription. That is, they may bind to negative glucocorticoid response elements that mediate the repression of gene transcription. However, phytochemical analysis of the twigs of the plant shows the presence of saponins, steroids, flavonoids, phenols,

terpenoids, and tannins. It is likely that the glucose lowering property could be due to the combined effect of these bioactive constituents. For instance, mollic acid glucoside, a compound isolated from *C. molle* leaf, possesses hypoglycaemic and antidiabetic properties in rodents [30]. Furthermore, phenolic constituents of *Pterocarpus marsupium* significantly lowered the glycemia of diabetic rats in a manner comparable to that of metformine [31]. DM is associated with profound alteration in the serum lipid and lipoprotein profile with an increased risk in coronary heart disease [32]. Hyperlipidemia is a recognized complication of DM characterized by elevated levels of cholesterol, triglycerides and phospholipids, and change lipoprotein composition [33]. Thus, reduction in TC and TG through dietary or hypocholesterolemic agent has been found beneficial in preventing cardiovascular disease risk factors (atherogenic and coronary artery indices) as well as improving lipid metabolism in diabetic patients [34]. In the present study, the increased TG, TC and LDL-c fraction and decreased HDL-c fraction were observed in diabetic rats. This abnormally variation of serum lipid levels is mainly due to uninhibited actions of lipolytic hormones on the fat depots, mainly due to impairment of insulin sensibility at diabetic state. After treated with *C. molle* extracts (250 and 500 mg/kg) or metformine (40 mg/kg) for 10 days, serum TG, TC and LDL-c levels were significantly decreased while serum HDL levels was increased; however, the increased HDL-c (cardioprotective lipid) level was comparable to the standard drug, metformine. Therefore, *C. molle* extracts have potential role to prevent formation of atherosclerosis and coronary heart disease. Several authors reported that secondary metabolites such as saponins, flavonoids, phenolic compounds, and triterpenoids have hypolipidemic activity [35]. Hence, the hypolipidemic properties of this plant may be due to different types of active secondary metabolites presents in the plant extracts. Moreover, in the liver, glucocorticoids increase the activities of enzymes involved in fatty acid synthesis and promote the secretion of lipoproteins. The hepatic lipogenic effect of glucocorticoids results in accumulation of triglycerides in the liver; this reduces insulin sensitivity in the liver [36]. The plant extracts could also act by inhibiting the expression of phosphoenolpyruvate carboxykinase and glucose-6-phosphatase and by increasing lipase activity in adipose tissue their by causing impairment of endothelium-dependent vasodilatation [37]. There exist a link between obesity, coronary heart disease and insulin resistance and weight loss has been reported to lower the risk of coronary heart disease and insulin resistance; in fact, weight loss of 5% or more has

been linked to improvements in glycaemic control, lipid parameters and quality of life [38]. In the present study, a severe loss in body weight observed in diabetic control might be the results of protein wasting to unavailability of carbohydrate for utilization as an energy source [39]. Metformine and AECM at dose of 500 mg/kg prevented this augmentation while the others dose of *C. molle* treated rats did not normalized the body weight completely. An increase in the body weight of diabetic treated rats might be due to an enhancement in glycaemic control and increased synthesis of structural proteins [40]. Moreover, a significant increase in liver and spleen weights was observed in non-treated diabetic rats. This increase of liver weight might be due to intensity metabolic activities in this organ, which is considered as detoxification organ. However, metformine and plant extracts prevented this increase in weight of organs.

CONCLUSION

From the present study, it can be concluded that, oral administration of twigs of *C. molle* as acute dose even 2000 mg/kg in albinos rats is relatively safe. Moreover, the hypoglycemic, antihyperglycemic, anti-hyperlipidemic and anti-atherogenic potentials of the extracts in type 2 DM model were confirmed. From preliminary phytochemical analysis it was found that the major chemical constituents of the *C. molle* extracts were glycosides, saponins, flavonoids, phenols, triterpenoids, and tannins so it is possible that certain of these constituents may be responsible for the observed antidiabetic activity and reduce lipid parameters and cardiovascular disease risk factors. If these results are extrapolated to humans, then *C. molle* might prove useful to be a source of potent type 2 DM and/or therapeutic principles acting also in preventing insulin resistance in non-diabetic states such as obesity and impaired glucose tolerance. This assertion lends credence to its suggested folkloric use in the control and/or management of insulin resistance DM in certain communities of Cameroun. Further pharmacological and biochemical investigations are underway to find out the active constituents responsible for antidiabetic activity and to elucidate its mechanism of action.

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Table 1: Effect of methanol and acetone extracts of *C. molle* on normoglycemic rats.

Groups	0 h	2 h	4 h
Control (10 mL/kg)	83.00 ± 1.51	82.33 ± 1.38	84.16 ± 1.01
Glibenclamide (0.3 mg/kg)	84.67 ± 1.54	72.83 ± 0.79**	75.67 ± 1.82*
MECM (250 mg/kg)	81.05 ± 1.61	80.00 ± 1.16	78.93 ± 1.67
MECM (500 mg/kg)	83.34 ± 1.50	76.17 ± 1.45	75.50 ± 1.93*
AECM (250 mg/kg)	83.33 ± 1.28	74.35 ± 1.68*	77.33 ± 1.64
AECM (500 mg/kg)	82.17 ± 1.87	74.33 ± 1.68*	75.50 ± 1.54*

Values are expressed as mean ± SEM (n = 6). *P < 0.05, ** P < 0.01 compared to control.

Table 2: Effect of *C. molle* extracts on body weight and relative organs weight in dexamethasone induced diabetic rats.

Groups	Body weight (g)		Organs weight (%)					
	0 th day	10 th day	Liver	Kidneys	Heart	Lungs	Spleen	Pancreas
Normal control	227.2 ± 11.53	259.20 ± 12.61	2.60 ± 0.07	0.55 ± 0.02	0.31 ± 0.02	0.61 ± 0.03	0.42 ± 0.03	0.32 ± 0.02
Diabetic control	221.8 ± 12.16	219.20 ± 13.07***	3.41 ± 0.20***	0.77 ± 0.03	0.44 ± 0.03	0.80 ± 0.06	0.87 ± 0.06**	0.36 ± 0.02
Dexa + MET (40mg/kg)	226.0 ± 7.46	248.20 ± 7.73b	2.97 ± 0.09*b	0.68 ± 0.01	0.60 ± 0.10	0.80 ± 0.07	0.67 ± 0.05	0.67 ± 0.05
Dexa + MECM (250 mg/kg)	218.4 ± 4.83	223.40 ± 3.85**	2.96 ± 0.09*b	0.64 ± 0.05	0.40 ± 0.03	0.75 ± 0.03	0.68 ± 0.06	0.30 ± 0.03
Dexa + MECM (500 mg/kg)	217.4 ± 7.16	228.00 ± 6.95*	2.46 ± 0.11c	0.61 ± 0.03	0.46 ± 0.03	0.68 ± 0.03	0.72 ± 0.06	0.27 ± 0.00
Dexa + AECM (250 mg/kg)	219.0 ± 3.96	227.80 ± 4.37*	2.80 ± 0.07c	0.56 ± 0.03	0.30 ± 0.01	0.63 ± 0.03	0.41 ± 0.03b	0.28 ± 0.02
Dexa + AECM (500 mg/kg)	222.0 ± 5.83	244.40 ± 5.10b	2.84 ± 0.03c	0.61 ± 0.02	0.34 ± 0.01	0.70 ± 0.05	0.47 ± 0.05b	0.35 ± 0.02

Values are expressed as mean ± SD of 6 rats in each group. *P < 0.05; ** P < 0.01; ***P < 0.001 compared to control. ^aP < 0.05, ^bP < 0.01; ^cP < 0.001 compared to diabetic control.

Table 3: Effect of *C. molle* on serum lipid and cardiovascular risk indices in dexamethasone-induced insulin resistance in rats

Groups	TC (mg/dL)	TG (mg/dL)	HDL-c (mg/dL)	LDL-c (mg/dL)	AI	CRI
Normal control	109.05 ± 2.74	81.70 ± 4.32	53.14 ± 2.46	39.57 ± 4.21	1.07 ± 0.10	2.08 ± 0.12
Diabetic control	127.03 ± 1.40***	101.79 ± 4.79***	34.91 ± 3.30***	71.76 ± 3.78***	2.77 ± 0.34***	3.76 ± 0.31***
Dexa + MET (40mg/kg)	100.95 ± 2.21c	76.05 ± 1.59c	53.88 ± 2.19c	31.85 ± 3.22c	0.87 ± 0.09c	1.89 ± 0.07c
Dexa + MECM (250 mg/kg)	117.57 ± 3.44a	89.87 ± 1.57c	50.65 ± 2.35c	48.94 ± 4.37c	1.34 ± 0.14c	2.43 ± 0.13c
Dexa + MECM (500 mg/kg)	106.90 ± 3.25c	80.36 ± 2.18c	54.48 ± 3.33c	36.33 ± 3.35c	0.98 ± 0.08c	1.89 ± 0.03c
Dexa + AECM (250 mg/kg)	102.70 ± 2.77c	83.68 ± 4.31c	49.72 ± 2.93b	36.60 ± 2.78c	1.11 ± 0.13c	2.11 ± 0.15c
Dexa + AECM (500 mg/kg)	110.27 ± 1.12c	77.49 ± 1.80c	54.68 ± 1.67c	40.09 ± 1.08c	1.02 ± 0.05c	2.00 ± 0.06c

Values are expressed as mean ± SEM (n = 6). *** P < 0.01 compared to control. ^aP < 0.01; ^bP < 0.01; ^cP < 0.001 compared to diabetic control.

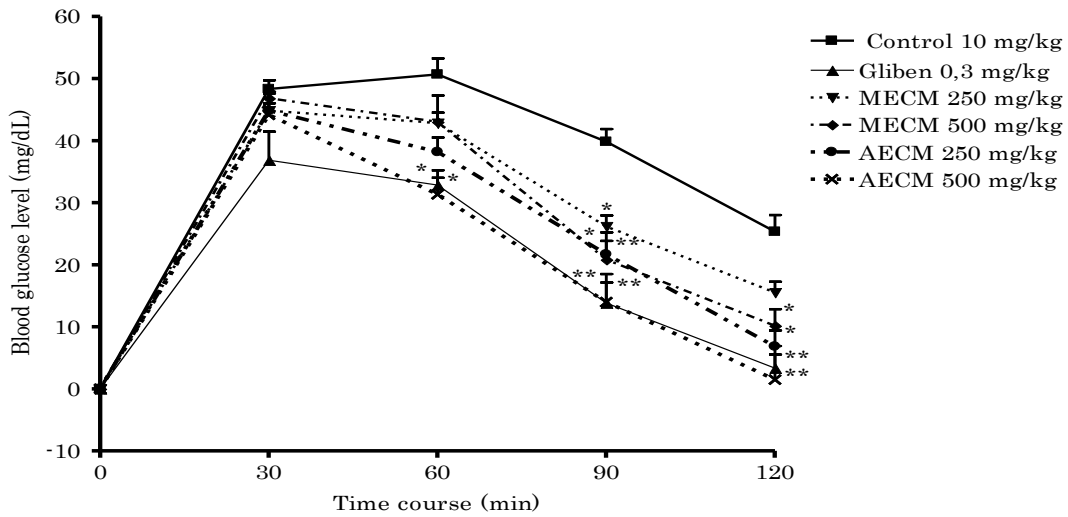


Figure 1: Effect of the extracts of *Combretum molle* on glucose tolerance test in normal rat.

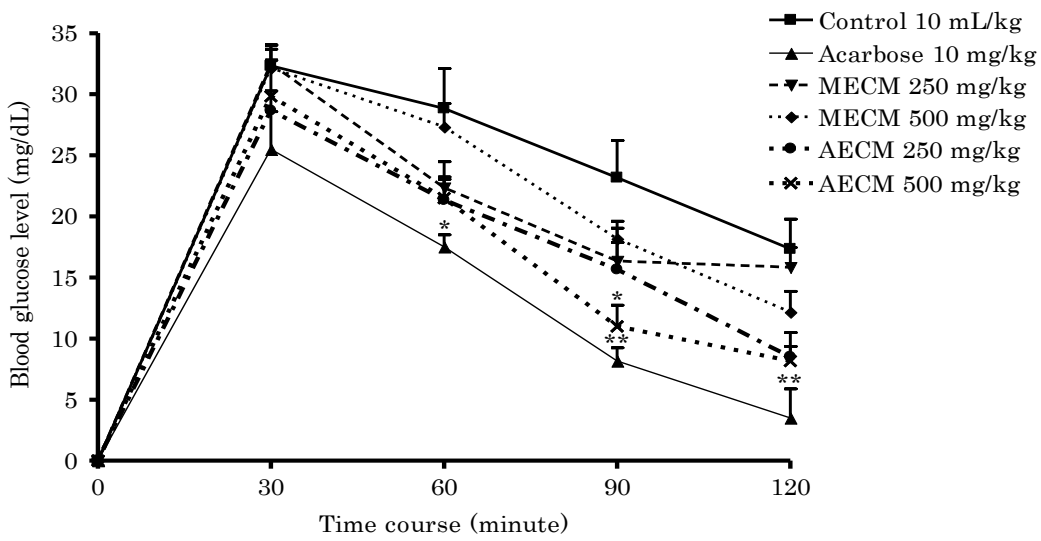


Figure 2: Effect of the extracts of *Combretum molle* on starch tolerance test in normal rat.

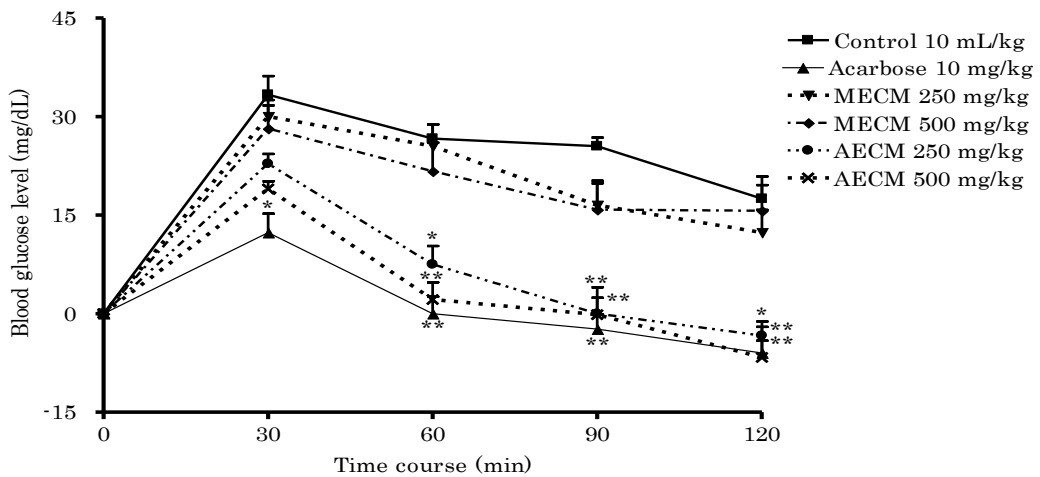


Figure 3: Effect of the extracts of *Combretum molle* on sucrose tolerance test in normal rat.

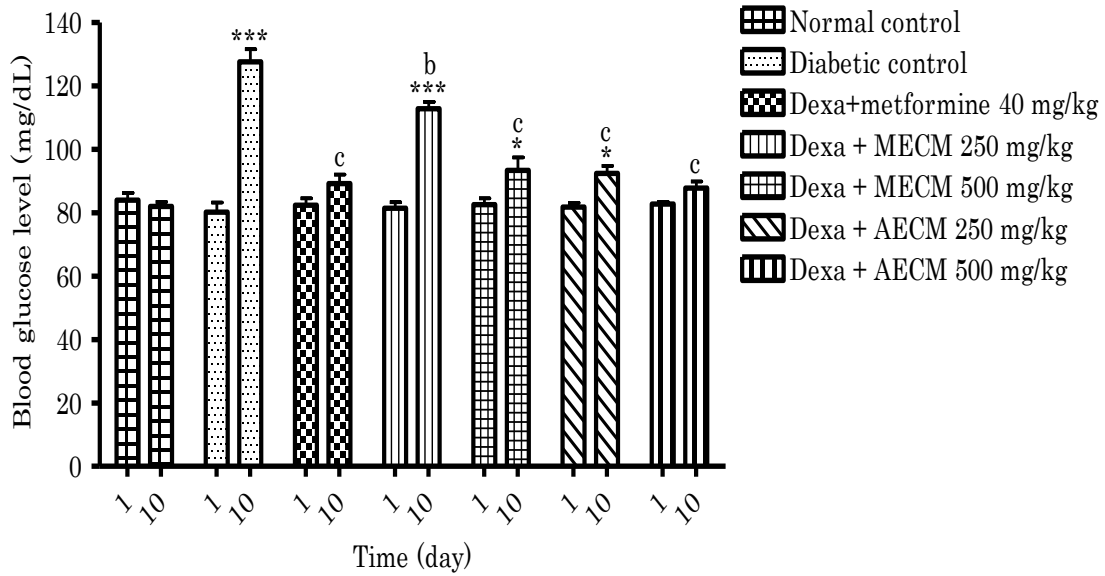


Figure 4: Effect of *Combretum molle* extracts on blood glucose level in rats.

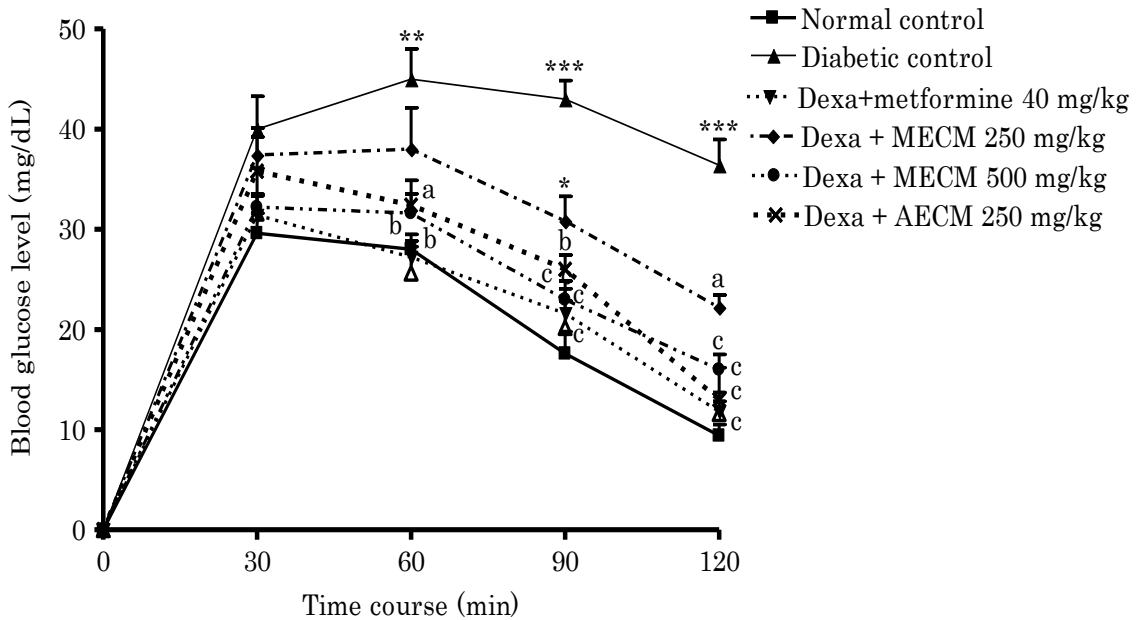


Figure 5: Effect of the extracts of *Combretum molle* on glucose tolerance test in diabetic rat.

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