INTRODUCTION

Hyperlipidemia refers to an abnormal increase in blood lipid levels, including triglycerides, total cholesterol, low-density lipoprotein (LDL) cholesterol. It is the main risk factor for the occurrence of cardiovascular disease. The diseases that are the leading cause of death worldwide and cause more than 17,000 deaths each year, or about 31% of total global mortality. According to the World Health Organization, hyperlipidemia presents itself following a rapid change in the eating habits and lifestyle of the population characterized by a diet increasingly rich in saturated fatty acids and a sedentary lifestyle. One of the most effective ways to manage hyperlipidemia is to reduce the absorption of fat, by inhibiting the activity of pancreatic lipase. Pancreatic lipase is an enzyme responsible for breaking down fats in the human digestive tract, converting the substrates of triglycerides found in edible fats to monoglycerides and free fatty acids, so that fats can penetrate and be absorbed by the small intestine.
Lipid-lowering drugs such as statins, fibrates, resins, tetrahydrolipstatin which is a special type of statin used to inhibit pancreatic lipase are used to manage hyperlipidemia but these drugs have harmful side effects such as kidney failure, sexual weakness and muscle pain.

The use of plants in herbal medicine is increasingly explored and many plants are known for their lipid-lowering effects, such as Camelia sinensis, Gardenia jasminoides and Basella alba. These plants, generally considered as lipid-lowering, all have similar characteristics: they are rich in bioactive molecules including flavonoids, tannins, saponins, phytosterols and many others. These molecules can act individually or in synergy in the management of hyperlipidemia by inhibition of certain enzymes involved in the biosynthesis of cholesterol and in the digestion of lipids. However, the mechanism by which this inhibition occurs varies with respect to the plant. In Cameroon, many plants have also been studied for their lipid and cholesterol lowering properties. Among these, the fruits of Dichrostachys glomerata have benefited in the last ten years from special attention as a lipid-lowering plant and this because of their high polyphenol content. Recent studies show, studies shown that biological activities of some plants depend on granulometric particles of their powders. In this logical research Deli et al., showed that the composition and antioxidant activities of fruits of Dichrostachys glomerata depends on particle size of the powder of this fruit. There is no study that presents antihyperlipidemic and hypolipidemic properties of fractions powder of fruits of Dichrostachys glomerata, therefore the aim of this work was to determine the lipid-lowering and anti-lipase properties of powder fractions of the fruits of Dichrostachys glomerata.

**MATERIAL AND METHODS**

**Plant material and production of D. glomerata powder fractions**

Dichrostachys glomerata fruits were bought from Mfoundi market in Yaounde (Central Region, Cameroon). Grinding and the sieving processes were used to obtain different powder fractions as describe by Deli et al. We obtained the following granulometric powder fractions: < 180 μm, 212 -180 μm, 315 -212 μm, ≥315 μm, unsieved powder.

**Extraction of lipase from rat’s pancreas**

Extraction was done as described by Shahani et al. 5g of freshly harvested rat pancreas were washed in physiological water and then placed in iced cold sucrose (1000mL; 0.01M) at 4°C. The pancreas was ground in a porcelain mortar and then homogenized in sucrose for 90s. The homogenate obtained was then centrifuged in eppendorf tubes at 15,000 g for 30 min in a refrigerated centrifuge. After centrifugation, the supernatant was subjected to precipitation with 50% (v / v) saturated ammonium sulphate (15 ml) with moderate stirring, left to stand for 30 min at 4°C, then centrifuged at 10,000 g for 30 min. The pellet obtained was then dissolved in a sucrose solution (20 mL; 0.01M) and again saturated with 50% ammonium sulfate. The mixture was centrifuged at 10000g for 30 min and the resulting pellet was dissolved in phosphate buffer (pH 7) and used as a source of enzyme.

**Determination of rat pancreatic lipase activity without aqueous extract of dichrostachys glomerata**

The inhibitory activity of pancreatic lipase in the absence of the extract was measured by titration with NaOH (0.02M). The activity of the pancreatic lipase of rats was determined by incubating an emulsion containing 8 ml of olive oil, 0.4 ml of phosphate buffer and 1 ml of the solution containing the pancreatic lipase of rats, for one hour and with shaking. Ultimately, 1.5 mL of a mixture containing acetone and 95% ethanol (v / v) was added to the mixture to stop the reaction. The amount of fatty acid released was determined by titration with NaOH (0.02M) using phenolphthalein as a color indicator. The equivalence point was observed with the appearance of a persistent pink color.

**Determination of rat pancreatic lipase activity with aqueous extract of dichrostachys glomerata**

The procedure is the same except that a quantity of 100 μl of a concentration range (1, 2.5, 5, 10, 15, 20, 25 mg / ml) of the aqueous extract of D. glomerata was added to the mixture. The appearance of a pink color pink compared to the yellow color showed released fatty acids, which was determined by titrating the solution against 0.02 M sodium hydroxide (0.02M) and using phenolphthalein as an indicator.

\[
A = \frac{(VT - V0) \times N \times NaOH}{V \times s \times 60} \quad B = \frac{(VDG - V0) \times N \times NaOH}{V \times s \times 60} \quad \% I = \frac{(A - B)}{A} \times 100
\]

A: Lipase activity without aqueous plant extract, B: Lipase activity with the aqueous plant extract, % I = Percentage inhibition of the fraction, V0: White volume (volume of NaOH obtained by titrating the substrate in the absence of lipase) (mL), VDG: Volume of NaOH in the presence of extract (mL), VT: Volume of NaOH without the extract (control) (mL), N: Normality of NaOH, VS: Volume of the solution before titration (11 mL), 60: Incubation time (minutes).

**Evaluation of antihyperlipidemic proprieties**

**Preparation of aqueous extract for animals**

The powder fractions were macerated in distilled water and with permanent stirring for 2 h and a volume of 10 ml / kg
was administered by gavage at a dose of 250 mg/kg of body weight of the animal.

**Diet formulation and animal experiments**

Male adult Wistar albinos rats (200-250 g) were obtained from the animal house of National School of Agro-Industrial Sciences of the University of Ngaoundere. The animals were used for both tests, antihyperlipidemic and hypolipidemic. Hyperlipidemia was induced by High Fat Diet (HFD) containing 300 g of egg yolk, 250 g of coconut oil and 50 g of soya oil, as described by Hamlat et al.18 with some modifications. Table 1 shows the different formulations.

48 Wistar albinos’ male rats weighing 200-250 g were randomly divided into 8 groups of 6 rats each. The rats were housed in cages in a room where the temperature was 37 °C and 12 h light and dark cycles were maintained and water was given ad libitum. The first group fed with normal diet + distilled water (normal control, CNo), the second group fed with HFD + distilled water (negative control, CN), the third group fed with HFD + atorvastatin (10mg/kg, positive control, CP), the fourth group was fed with HFD + powder fraction 315 – 212 µm of D. glomerata (F1), the fifth group was fed with HFD + powder fraction 212 – 180 µm of D. glomerata (F2), the sixth group was fed with HFD + powder fraction < 180 µm of D. glomerata (F3), the seventh group was fed with HFD + unsieved powder of D. glomerata (FN), all powder fractions were given at a dose of 250mg/kg of body weight. The composition of the diets is shown in Table 1. The animals were weighed each week, the quantity of feed intake was obtained by subtracting the remaining feed from the quantity administered the previous days. After four weeks, rats were fasted for 14 h, anaesthetized by inhalation of diethyl ether impregnated on a cotton wool, and blood was collected via cardiac puncture. The liver, kidneys, brain, testicles and heart were removed. For each rat sample weighed, the organs liver, kidneys, brain, testicles and heart were also weighed and the ratio of organ to body weight calculated. The blood was collected in the dry tubes and submitted to centrifugation at 3500 rpm for 15 min to obtain serum.

**Biochemical evaluation**

The serum samples were used to quantify some biochemical parameters. Total cholesterol (CT), triglycerides (TG) and High-density lipoprotein cholesterol (HDL-C) were evaluated using enzymatic kits (HUMAN kits) according to procedures described by Richmond19 and Graphocal.20 Low density lipoprotein cholesterol (LDL-C) was then calculated according to Friedewal et al.’s formula.21

**Evaluation of hypolipidemic proprieties**

**Animal experiments**

During the antihyperlipidemic test, 24 Wistar albinos male rats weighing between 200-250 g were divided into 4 groups of 6 rats each. They were also fed with high fat diet for four weeks to induce hyperlipidemia. After induction these rats were used for hypolipidemic test during which the rats were fed with the normal diet for four weeks. The first group fed with normal diet + powder fraction 212 – 180 µm of D. glomerata (FT) at a dose of 250mg/kg of body weight, the second group fed with normal diet + atorvastatin (10mg/kg, positive control, CP), the third group fed with normal diet + distilled water (negative control, CN) and the normal control group (CNo) which is the group which during the induction of hyperlipidemia was fed with normal diet and continued with normal diet in hypolipidemia test + distilled water. After four weeks of experimentation, the animals were sacrificed, and the different parameters were evaluated as described in antihyperlipidemic test. The powder fraction 212 – 180 µm used for hypolipidemic properties was justified by its highest antioxidant properties shown by studies of Deli et al.15

**Analysis of faecal lipid**

Feces were collected daily for the evaluation of the excreted lipids. The fecal matter was then dried and crushed with the porcelain mortar into fine powder. The total lipids were extracted using the Soxhlet apparatus, according to the Russian method described by Bourely.22

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**Table 1: Formulation of diets for rats**

<table>
<thead>
<tr>
<th>Food components</th>
<th>Normal diet</th>
<th></th>
<th>High fat diet</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Incorporation (g/kg)</td>
<td>Energy (Kcal)</td>
<td>Incorporation (g/kg)</td>
<td>Energy (Kcal)</td>
</tr>
<tr>
<td>Proteins</td>
<td>Fish powders</td>
<td>200</td>
<td>800</td>
<td>140</td>
</tr>
<tr>
<td>Glucides</td>
<td>Starch</td>
<td>590</td>
<td>2360</td>
<td>250</td>
</tr>
<tr>
<td></td>
<td>Sucrose</td>
<td>50</td>
<td>200</td>
<td>50</td>
</tr>
<tr>
<td>Lipids</td>
<td>Coconut oil</td>
<td>-</td>
<td>-</td>
<td>250</td>
</tr>
<tr>
<td></td>
<td>Egg yolk</td>
<td>-</td>
<td>-</td>
<td>300</td>
</tr>
<tr>
<td>Others</td>
<td>Soybean oil</td>
<td>50</td>
<td>450</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Cellulose</td>
<td>50</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Minerals</td>
<td>10</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Vitamins</td>
<td>50</td>
<td>-</td>
<td>50</td>
</tr>
<tr>
<td>Total</td>
<td>1000</td>
<td>3810</td>
<td>1000</td>
<td>7160</td>
</tr>
</tbody>
</table>
Statistical analysis
The statistical analyses were performed using the Statgraphics software, version 5.0. The values were presented as means with their standard deviation (± SD). One-way analysis of variance (ANOVA) was performed to test the significant differences (P < 0.05) between groups. When the difference was significant, a Duncan multiple comparison range test was used as a post hoc test.

RESULTS

Antihyperlipidemic properties of powder fractions of D. glomerata

Effects of powder fractions of D. glomerata on the body weight and the food intake of rats

Food intake and weight gain of rats at the end of antihyperlipidemic test is shown in Table 2, food intake between all group is statistically equal (p>0.05), but weight gain is different. Weight gain in normal control groups is the lowest, and in negative control group is the highest. In the groups which took powder fractions, weight was low compared to the negative control group (p < 0.05), and the groups which consumed unsieved powder had the lowest value.

Effects of powder fractions of D. glomerata on lipid profile of rats

Figure 1 shows the effect of powder fractions of D. glomerata on total cholesterol and LDL-Cholesterol, this result shows that atorvastatin and powder fractions of D. glomerata reduce levels of total cholesterol and LDL-Cholesterol. levels of total cholesterol and LDL-Cholesterol of negative control group are statistically high compared to those which consumed powder fractions. Groups which consumed powder fractions 212 – 180 μm and < 180 μm, present the lowest value (p < 0.05). Same observation was noted with triglycerides levels as shown in Figure 2. Concerning HDL-Cholesterol, in Figure 2 we see that atorvastatin and powder fractions of D. glomerate increase levels of HDL-Cholesterol, increases were significantly important in groups which consumed powder fractions 315 – 212 μm, 212 – 180 μm, < 180 μm and unsieved powder (p < 0.05).

Effects of powder fractions of D. glomerata on organ-to-body weight ratios of rats

As shown in Table 3, all the organ-to-body weight ratios of groups of rats except liver, is statistically equal (p>0.05).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Weight gain (g)</th>
<th>Food intake (g/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNo</td>
<td>14.26±0.56*</td>
<td>25.72±2.12*</td>
</tr>
<tr>
<td>CN</td>
<td>40.21±2.62*</td>
<td>24.57±3.06*</td>
</tr>
<tr>
<td>CP</td>
<td>20.32±3.53*</td>
<td>24.86±2.65*</td>
</tr>
<tr>
<td>F1</td>
<td>21.25±2.01*</td>
<td>25.07±2.95*</td>
</tr>
<tr>
<td>F2</td>
<td>25.03±3.01*</td>
<td>24.77±3.10*</td>
</tr>
<tr>
<td>F3</td>
<td>23.25±2.09*</td>
<td>23.98±4.12*</td>
</tr>
<tr>
<td>F4</td>
<td>25.55±3.53*</td>
<td>25.4±2.54*</td>
</tr>
<tr>
<td>FN</td>
<td>17.05±1.50*</td>
<td>24.82±2.95*</td>
</tr>
</tbody>
</table>

Table 2: Food intake and weight gain of rats at end of antihyperlipidemic test

CNo: normal control, CN: negative control, CP: positive control, F1: powder fraction ≥ 315 μm, F2: powder fraction 315 – 212 μm, F3: powder fraction 212 – 180 μm, F4: powder fraction < 180 μm, FN: unsieved powder. Mean ± SD followed by different letters (a–d) in the same column are significantly different (p<0.05).

Figure 1: Total cholesterol and LDL-Cholesterol levels of rats fed with powder fractions of D. glomerata

Concerning the liver, negative control group has the highest value, this is could be due to the accumulation of fat in the liver since it is the group that taken just high fat diet.

### Hypolipidemic proprieties of powder fractions 212 – 180 µm of D. glomerata

#### Effect of powder fraction 212 – 180 µm of D. glomerata on the body weight, the food intake of rats and faecal lipid

As shown in Table 4, there are no significant differences (p>0.05) in food intake of all groups. Weight gain in normal control group, negative control group and positive control group is statistically equal but high in the group which consumed powder fraction 212 – 180 µm, in this group we note a decrease in weight (p < 0.05). It appears from this table that percentage of faecal lipid of group taken powder fraction 212 – 180 µm is statistically higher than that of others groups which are identical. This increase in the lipid level in the feces of rats which consumed fractions of D. glomerata could be explained by the bioactive compounds present in the fraction which would have acted by preventing the absorption of lipids, hence their elimination in the feces.

#### Effect of powder fraction 212 – 180 µm of D. glomerata on organ-to-body weight ratios of rats

Table 5 shows the organ-to-body weight ratios of rats after four weeks of treatment, these results show that there was no significant difference between value of organ-to-body weight ratios of kidney, pancreas, testis, lungs and heart of rats of all groups (p>0.05), however value of liver of normal control and group which consumed powder fraction are not different but low compared to those of others groups. This result can be
explained by the fact that the fraction could have no deleterious effect on the organs.

Effect of powder fractions 212 – 180 µm of D. glomerata on lipid profile of rats

Table 6 shows that administration of powder fraction 212 – 180 µm of D. glomerata to FT group and atorvastatin to CP group for four weeks resulted in a significant (p < 0.05) decrease in the total cholesterol, triglycerides, LDL-Cholesterol levels and increase in HDL-Cholesterol of the rats of these groups compared to control negative group.

Anti-lipase activity of the aqueous extract of powder fraction 212 – 180 µm of D. glomerate

Figure 3 shows that the lipase activity is very affected by the extract of the plant fraction. Indeed, this activity is weaker at high concentration; it is 0.15 Mmol/ml/min for a concentration of 25 mg / mL; 0.29 Mmol/ml/min for 20 mg / mL; 0.33 Mmol/ml/min for 15 mg / mL; 0.39 Mmol/ml/min for 10 mg / mL, 0.42 Mmol/ml/min for 5 mg / mL; 0.5 for 2.5mg / mL; 0.54 Mmol/ml/min per 1 mg / mL and 0.67 Mmol/ml/min for 0 mg / mL.

At the concentration of 0 mg/ml, lipase activity is without extract. The percentage of inhibition of pancreatic lipase activity by the extract of the 180-212 µm fraction of D. glomerata describes an exponential appearance depending on the concentration of the extract. The highest inhibition percentage (86.43%) was obtained for the 25 mg / mL concentration. The IC50 which is the concentration which inhibits 50% of the lipase was determined by extrapolation from the curve of the percentages of inhibitions (Figure 4) and is 5 mg/ml. It is important to remember that the smaller the IC50, the greater the lipase inhibition. the lower IC50 value is explained by the inhibitory action of the high phenolic compounds contained in the 180-212 µm fraction.

DISCUSSION

Concerning antihyperlipidemic test, the decrease in weight gain observed in groups which consumed powder fractions may be due to the ability of D. glomerata to reduce

Table 4: Food intake, weight gain/ lost and faecal lipid of rats at end of hypolipidemic test

<table>
<thead>
<tr>
<th>Groups</th>
<th>Weight gain/ lost (g)</th>
<th>Food intake (g/day)</th>
<th>Faecal lipid (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNo</td>
<td>8.26±1.23a</td>
<td>23.72±2.50a</td>
<td>7.66±1.02a</td>
</tr>
<tr>
<td>CN</td>
<td>8.35±1.71a</td>
<td>22.57±2.22a</td>
<td>9.32±2.11a</td>
</tr>
<tr>
<td>CP</td>
<td>9.50±2.07a</td>
<td>22.86±2.43a</td>
<td>8.64±2.05a</td>
</tr>
<tr>
<td>FT</td>
<td>-12.05±2.52b</td>
<td>23.82±2.67a</td>
<td>14.97±2.10a</td>
</tr>
</tbody>
</table>

Table 5: Organ-to-body weight ratios of rats at end of hypolipidemic test

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver</th>
<th>Kidney</th>
<th>Pancreas</th>
<th>Testis</th>
<th>Lungs</th>
<th>Heart</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNo</td>
<td>3.32±0.06a</td>
<td>0.57±0.09a</td>
<td>0.64±0.19a</td>
<td>0.46±0.64a</td>
<td>0.53±0.02a</td>
<td>0.29±0.10a</td>
</tr>
<tr>
<td>CN</td>
<td>4.43±0.53b</td>
<td>0.61±0.10b</td>
<td>0.81±0.20b</td>
<td>0.82±0.02a</td>
<td>0.59±0.03a</td>
<td>0.48±0.02b</td>
</tr>
<tr>
<td>CP</td>
<td>4.52±0.21b</td>
<td>0.73±0.09b</td>
<td>0.90±0.07a</td>
<td>1.12±0.05a</td>
<td>0.56±0.10a</td>
<td>0.40±0.10a</td>
</tr>
<tr>
<td>FT</td>
<td>3.45±0.31a</td>
<td>0.93±0.08a</td>
<td>0.91±0.06a</td>
<td>1.01±0.20a</td>
<td>0.62±0.02a</td>
<td>0.39±0.10a</td>
</tr>
</tbody>
</table>

Table 6: Total cholesterol, triglycerides, HDL and LDL cholesterol levels of rats fed with powder fractions 212 – 180 µm of D. glomerate

<table>
<thead>
<tr>
<th>Groups</th>
<th>Triglyceride</th>
<th>Total cholesterol</th>
<th>HDL-cholesterol</th>
<th>LDL-cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNo</td>
<td>136.45 ± 3.61b</td>
<td>108.00 ± 0.92b</td>
<td>63.15 ± 1.77b</td>
<td>17.56 ± 0.06b</td>
</tr>
<tr>
<td>CN</td>
<td>193.70 ± 3.10b</td>
<td>127.00 ± 0.57b</td>
<td>41.20 ± 0.71b</td>
<td>47.06 ± 1.89b</td>
</tr>
<tr>
<td>CP</td>
<td>144.40 ± 2.26b</td>
<td>103.55 ± 0.92b</td>
<td>58.80 ± 0.71b</td>
<td>15.87 ± 1.17b</td>
</tr>
<tr>
<td>FT</td>
<td>127.40 ± 0.99b</td>
<td>109.65 ± 0.92b</td>
<td>70.15 ± 1.06b</td>
<td>14.02 ± 0.06b</td>
</tr>
</tbody>
</table>
fat absorption and lipogenic enzymes and increase fat excretion. In fact, D. glomerata fruit contains polyphenols which can inhibit pancreatic lipase activity and lipid absorption thus helps to manage weight. Reduction of blood lipid levels observed could be explained by polyphenols (flavonoids, tannins) contained in powder fractions of D. glomerata. In fact, polyphenols could inhibit pancreatic lipase and facilitate excretion of triglycerides, polyphenols could also form complexes with cholesterol and bile acids and cause their excretion in feces, reduction of HMG-CoA reductase activity by polyphenols is another possible mechanism.

Concerning hypolipidemic test, significant reduction in the body weight of rats treated with the 180-212 μm fraction of D. glomerata powder could be explained by its high content of bioactive compounds. This result is in agreement with the study by Kuete et al. which shows a reduction in body weight in obese individuals (30-40 kg/m²) after treatment (8 weeks) with 200 mg of D. glomerata. Several studies have reported that the reduction in weight gain is associated with the consumption of foods rich in phenolic compounds which act according to several mechanisms including: the suppression of fat absorption by the intestine, the inhibition of differentiation from pre-adipocytes to adipocytes and stimulation of apoptosis of, mature adipose tissues. Reduction of levels of blood lipids could be also due to the bioactive compounds in the 180-212 μm fraction of D. glomerata. These bioactive compounds can act according to several mechanisms: the modification of lipoprotein metabolism by reducing plasma triglycerides and Apo B concentrations, the reduction of cholesterol absorption by interaction with cholesterol transporters present on the membrane of certain intestinal cells, complexation of bile acids (necessary for the synthesis of cholesterol).

Another possible mechanism of bioactive compounds may be the inhibition of pancreatic lipase. Several authors have reported that polyphenols have the ability to inhibit pancreatic lipase, an enzyme that ensures the hydrolysis of triglycerides into monoglycerides, diglycerides and free fatty acids to be absorbed by the intestine. The inhibition will cause the malabsorption of lipids in general and those of triglycerides in particular and consequently their excretions and their plasma levels decrease. Lipase activity is very affected by the extract of the plant fraction, this result can be explained by the fact that polyphenols can bind to pancreatic lipase and modify its structure. Zaidi also found dose-dependent activity using extracts from Rhamnus alaternus leaves.

CONCLUSION

Results obtained from the present study indicate that powder of fruits of D. glomerata have significant antihyperlipidemic activities which depend on powder fractions. Fraction 180-212 μm exhibits hypolipidemic effect. Inhibition of pancreatic lipase is one of mechanisms which could explain the effects obtained.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the technical assistance of the laboratory staff from the Department of Food Science and Nutrition of National School of Agro-industrial Sciences of Ngaoundere University.

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