**Research Article**

**Effect of aqueous extract of Clerodendrum thomsoniae Linn. leaves on the onset of hyperlipidaemia and the inhibition of gain mass on wistar rats**

Deutchoua Ngounou Eric Martial¹, Mang Yannick Dimitry², Dongmo Faustin¹, Youdom Patrick¹, Sokeng Dongmo Sélestin¹, Njintang Yanou Nicolas³

¹Department of Biological Sciences, Faculty of Science, University of Ngaoundere, PO Box 454, Ngaoundere-Cameroon
²Department of Tourism and Hotel Management, Higher Technical Teacher's Training College of Ebolowa, University of Yaoundé I, PO Box 886, Ebolowa-Cameroon
³Department of Food Science and Nutrition, Physical and Chemistry Food Laboratory, ENSAI, University of Ngaoundere, PO Box 455, Ngaoundere-Cameroon

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Abstract

**Background:** The genus Clerodendrum is among the most important in the plants' kingdom with many species known for their medicinal properties and among which Clerodendrum thomsoniae is well represented. Previous studies established hypolipidemic, antioxidant and antidiabetic properties of aqueous extract (AECT) of Clerodendrum thomsoniae. **Objective:** This study aimed at evaluating the effect of the AECT on the onset of hyperlipidaemia in wistar rats. **Material and methods:** Normal Wistar rats were administered AECT (312.5, 625 and 1250 mg/kg, p.o.) followed by feeding with high fat diet for five weeks. Lipid parameters such as total cholesterol (TC), Low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDLC) and triglycerides (TG) levels were measured in serum. The modifications in the body mass, feed intake were evaluated. The results revealed that HFD induced an alteration in serum, lipid profile (triglyceride, cholesterol, HDL, and LDL) in negative control rats. Meanwhile in rats subjected to AECT (312.5, 625 and 1250 mg/Kg p.o) no significant changes were noticed with the normal control rats (p < 0.05). On the other hand, concerning body mass, no significant change was observed between the normal control and test rats. AECT showed the ability to prevent the onset of hyperlipidaemia in rats. Hence, it could be used in the management of cardiovascular diseases, obesity and metabolic syndrome.

**Keywords:** Clerodendrum thomsoniae, high fat diet, Cholesterol, hyperlipidaemia, antidiabetic

Introduction

Hyperlipidaemia which is the most common form of dyslipidaemia (Fredrickson,1965) is defined as a chronic qualitative and quantitative change in lipid metabolism characterized by an increase in blood levels of certain lipid parameters such as triglycerides, total cholesterol and LDL as well as reduced high-density lipoprotein cholesterol level (Taskinen, 2003; Thiombiano et al., 2016; Ahmad et al., 2018). In its course, hyperlipidaemia associates diseases such as: cardiovascular diseases (CVD), Obesity, metabolic syndrome and even diabetes (Whitfield, 2017). The close relationship between hyperlipidaemia and cardiovascular diseases has been well documented (Ma et al., 2012; Daniels, 2012). Obesity is one of the most common global health problems characterized by accumulation of fat (triglycerides and cholesterol) in adipocytes (Sinha et al., 2002; Weiss et al., 2004; Farag Gaballa, 2011). Excess calories, alcohol and sugar in the body get converted into triglycerides and stored in fat cells throughout the body (Smelt, 2010). Cholesterol is a vital component of the mammalian cell membrane of all tissues and is a precursor of steroid hormones and bile acids (Verma, 2017). Dietary supplements containing antioxidants and hypolipidaemic agents may be useful to prevent and manage...
obesity and related complications (Shreja and Mir, 2017). Dietary supplements are good alternative to weight-loss drugs frequently associated with one or more side effects, which are sometimes very harmful for the treatment of various diseases such as metabolic syndrome, diabetes, and cancer.

In addition to dietary supplements, medicinal plants have always been considered as a healthy source of life for all people due to its rich therapeutic properties and being 100% natural (Edeoga et al., 2005). Medicinal plants are widely used by the majority of populations to cure various diseases and illnesses, which have a high impact on the world’s economy (Bauman, 2004; Cumali Keskin, 2018). Herbal drugs and products used in traditional medicine have been reported to control and manage hyperlipidemia and weight (Vermaak et al., 2011; Xinyu Ji et al., 2019). In Africa and in Cameroon one of the plants used in traditional medicin to manage disorder of lipids parameters, hyperglycaemia is Clerodendrum thomsoniae. Various studies have established its hypolipidemiant, antioxidant properties (Deutchoua et al., 2020a), and antidiabetic properties (Ngounou et al., 2021) and its toxicity (Deutchoua et al., 2020b). Lipid profile management is one of the most successful strategies for the management of obesity (Anderson et al., 2012). The present investigation involved evaluation of the effect of aqueous extract of the leaves of Clerodendrum thomsoniae Linn leaves on the onset of hyperlipidaemia in wistar rats.

Materials and methods

Plant material and production of powder

Plant material used for this study was obtained from Mbideng (Latitude: 7°31'05.54"; N7°18'37.99''512", Longitude :13°35'29.90832''), a neighborhood of Ngaoundere, in the Adamawa Region of Cameroon. For the production of C. thomsoniae powder, mature leaves of the plant were carefully cleaned, sorted, graded according to size and dried in a ventilated electric turning dryer (brand Riviera & Bar) at 40 ± 2°C for 48 h. After drying, the leaves were ground to fine powder using an electric grinder (Culatti, Polymix, France) equipped with a sieve of diameter 500 μm mesh. The obtained powder was directly used for production of the aqueous extract.

Preparation of aqueous extract of Clerodendrum thomsoniae

The powder (2.5 g) was blended with 40 mL distilled water. The different mixtures were placed in a water bath at 70 ± 2°C for 30 min while being stirred. The mixture was then cooled for 30 min and centrifuged at 1500rpm for 15 min at 20°C using refrigerated centrifuge. The supernatant was collected and the residue was solubilized in 40 mL and re-extracted as mentioned above. The supernatants were combined and concentrated under vacuum in a rotary evaporator and dried in a desiccator at 40°C. The crude extract was weighed and used to prepare solutions of concentrations 312.5, 625 and 1250 mg/mL representing the 3 test aqueous extracts of C. thomsoniae.

Experimental Animals

The study was conducted with adult and healthy male Wistar rats (weighing 150 - 200 g). They were procured from the animal house of the Faculty of Science, Ngaoundere University, Cameroon. The animals were kept in cages, 1 per cage, with relative humidity (54±2 %) in a 12 hrs light/dark cycle at 25 ± 2°C. They were given access to water and a standard diet before experimentation. The experiment was carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and ethically approved by the Institutional Committee of the Ministry of Scientific Research and Innovation of Cameroon (00 (237) 699870979).

The high fat diet was prepared and administered for 5 weeks except for the normal control group that were fed with standard diet. The compositions of the two diets were as presented in the table 1.

<table>
<thead>
<tr>
<th>Table 1. Content of standard and high fat diet (g/kg) (Hamlat et al., 2008)</th>
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<tbody>
<tr>
<td><strong>Contents</strong></td>
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<tr>
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<tr>
<td>Proteins</td>
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<td>Glucids</td>
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<td>Others</td>
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<td>Total</td>
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In vivo assessment of antihyperlipidaemic activity

The principle of this experiment was based on the ability of the AECT to inhibit the onset of hyperlipidaemia. Thirty normal healthy Wistar male rats weighing 150 - 200 g were randomly divided into 6 groups of 5 rats each. The rats were kept 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 standard diet (normal control), the negative control group received distilled water at a dose of 10mL/kg and fed with high fat diet (HFD); the test group received daily AECT at respective doses (312.5 mg/kg, 625 mg/kg and 1250 mg/kg) and fed with HFD. The positive control group received atorvastatin at 5 mg/kg and fed with HFD. 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. Feces were collected daily for the evaluation of the excreted lipids. After five weeks, the animals were sacrificed by anesthesia using diethyl ether, and the blood samples were collected from the jugular vein. The blood was collected in the dry tubes and allowed to stand for 30 min, then centrifuged at 3000 trs/min for 15 min, serum samples obtained were collected in dry tubes, and kept at -20 °C for biochemical estimation.

Blood sampling and biochemical analysis

At the end of 5 weeks treatment of rats as described above, the rats were overnight-fasted (12 hrs), anaesthetized by inhalation of isoflurane impregnated on a cotton wool and sacrificed. Analysis of serum for total cholesterol (TC), Triglycerides, High-density lipoprotein cholesterol (HDL-c), Very low-density Lipoprotein cholesterol (VLDLc) and Low-density Lipoprotein cholesterol (LDLc) was performed using commercial kits «Human Kits» and following standard procedures outlined by Aissatou et al. (2017).

Analysis of faecal lipid

Feces were collected daily for the evaluation of the excreted lipids. The faecal matter was then dried and crushed with the porcelain mortar into fine powder (Aïssatou et al., 2017). The total lipids were extracted using the Soxhlet apparatus, according to the Russian method described by Bourely (1982).

Results

Effects of the aqueous extract of the leaves of C. thomsoniae on the body mass, the feed intake of the rats

Figure 1 shows that there is a significant difference between the feed intake of all groups of rats after five weeks of treatment. There is significant difference between the groups that received extract (GIII 312.5 mg/Kg, GIV 625 mg/Kg and GV 1250 mg/Kg), the group that received reference (GVI Atorvastatin 5mg/Kg) and the groups that did not received (GI and GII distilled water). The observations and analysis made standout; a significant increase in feed intake in all the groups compared to the group that received standard diet (normal control).

Concerning changes in mass gain/mass loss, no loss in body mass has been observed. All the groups presented gain in body mass with an important increase of about 78.55% of the initial mass in rats of negative control group (GII) that received distilled water as treatment, a significant difference (P<0,05) with other groups of rats. Meanwhile, there is no significant difference between the groups that...

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Figure 1. Effect of the aqueous extract of the leaves of C. thomsoniae on body mass and food intake of the rats
received AECT at different doses (GIII, GIV and GV), reference (GVI) with the normal control that was fed with standard diet despite gain mass observed five weeks later after treatment.

Furthermore, it appears that the increase in body mass is proportional to the feed intake in group II rats (Negative Controls) treated with distilled water as opposed to the groups of rats treated with either the different doses of the extract or the reference drug.

**Effects of the aqueous extract of the leaves of *C. thomsoniae* on creatinin, glucose levels of rats**

Compared to the normal control (GI), high fat diet did not bring significant changes (P < 0.05) in the serum levels of creatinine in other groups (Figure 2).

Concerning the level of glucose and compared to the normal control (GI) a significant increase has been observed (P < 0.05) in glucose level. Meanwhile, a decrease in glucose level has been observed in treated groups (GIII, IV, V and VI) compared to the negative control (GII) receiving distilled water as treatment. No significant difference was observed between the normal control and the rats treated with AECT at respective doses of 625 mg/Kg and 1250 mg/Kg. These doses brought a decrease in level of glucose more than the reference (Atorvastatin).

**Table 2. Effect on faecal lipids excretion**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal control</th>
<th>Negative control</th>
<th>AECT 312.5+HFD</th>
<th>AECT 625+HFD</th>
<th>AECT 1250+HFD</th>
<th>Positive control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid</td>
<td>6.22±0.21</td>
<td>4.35±0.19</td>
<td>10.97±0.24</td>
<td>13.35±0.18</td>
<td>15.27±0.17</td>
<td>12.47±0.21</td>
</tr>
</tbody>
</table>

AECT = Aqueous extract of *Clerodendrum thomsoniae* at different doses (312.5; 625 et 1250mg/Kg). HFD=High Fat Diet. Data expressed as Mean ± SEM (standard error of mean); n =5. In the same line means with different letters are significantly different (P<0.05).

**Table 3. Effects of AECT on serum lipid profile**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal Control</th>
<th>Negative Control</th>
<th>AECT 312.5+HFD</th>
<th>AECT 625+HFD</th>
<th>AECT 1250+HFD</th>
<th>Positive Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL mg/dL</td>
<td>32.3±1.1b</td>
<td>31.1±1.5b</td>
<td>31.9±1.3b</td>
<td>36.5±0.9c</td>
<td>40.9±2.5d</td>
<td>32.4±1.8b</td>
</tr>
<tr>
<td>LDL mg/dL</td>
<td>29.7±0.8a</td>
<td>66.5±1.8b</td>
<td>48.6±1.6c</td>
<td>31.6±1.7d</td>
<td>21.2±2.4d</td>
<td>20.3±1.5e</td>
</tr>
<tr>
<td>VLDL mg/dL</td>
<td>20.5±1.1c</td>
<td>25.2±1.3c</td>
<td>19.2±0.4c</td>
<td>13.3±3.8a</td>
<td>11.2±2.4c</td>
<td>17.4±2.6c</td>
</tr>
<tr>
<td>CT mg/dL</td>
<td>84.7±0.3c</td>
<td>122.5±1.6b</td>
<td>89.4±1.2d</td>
<td>75.5±4.2b</td>
<td>70.2±4.9e</td>
<td>70.5±6.7a</td>
</tr>
<tr>
<td>TG mg/dL</td>
<td>100.7±0.6c</td>
<td>125.7±5.4c</td>
<td>95.7±4.2d</td>
<td>68.1±1.6b</td>
<td>65.9±1.2a</td>
<td>86.8±3.3c</td>
</tr>
</tbody>
</table>

AECT = Aqueous extract of *Clerodendrum thomsoniae* at different doses (312.5; 625 et 1250mg/Kg). HFD=High Fat Diet. Data expressed as Mean ± SEM (standard error of mean); n =5. In the same line means with different letters are significantly different (P<0.05).
Compared to the negative control, groups GII, GIV and GV excreted more lipids. The rats receiving AECT1250 (GV) excreted more lipids (Table 2) than the rats receiving the reference-Atorvastatin (GVI) thus showing that AECT inhibited absorption of lipids.

HFD induced hyperlipidaemia in rats receiving distilled water and lead to an increase in plasma TC, TGs, LDL, VLDL as shown in Table 2. In addition, atorvastatin and aqueous extract of *C. thomsoniae* reduced lipid parameters. In fact, levels of TC, TG and LDL-C of rats belonging to the negative group (GI) are higher than that of the groups taking AECT (312.5 mg/kg GIII, 625 mg/kg GIV and 1250 mg/kg GV) and the group that received atorvastatin (GVI). The contrary was obtained with HDL-C. AECT in test groups at the dose of 625 and 1250 mg/kg for five weeks raised level of High-density Lipoproteins-cholesterol more important than the reference.

**Statistical Analysis**

The experiments for which data is reported in the tables and figures were carried out in triplicate or more replicate determinations. All data were expressed as mean ± standard deviation and were statistically analyzed using one-way analysis of variance (ANOVA). When statistical differences were found, the Duncan's Multiple Range Test was applied in order to classify samples at the significance level of 5%. The Statgraphics Program (Statically Graphics Educational, version 6.0, 1992, Manugistics, Inc. and Statistical Graphics Corp., USA) was used for the statistical analysis.

**Discussion**

In this study, the inhibitory onset of the hyperlipidaemia in wistar rats was investigated for the first time. The decrease in the plasma of total cholesterol and triglyceride levels may be attributed to the presence of hypolipidaemic agents in the extract. The general lack of significant changes in HDL and LDL levels indicate that the extract had no effect on lipid metabolism of animals.

We studied the preventive effect of AECT on the accumulation of fat. Many types of diet as source of lipids are used in experiments to study physiopathology and the treatment of dyslipidaemia on animal models. As example, we have: diet riched in lard (Vijaya et al., 2009), in lin oil (Darimont et al., 2004), in cholesterol and coconut oil (Hamlat et al., 2008). The latter was used in this work. The results in figure 1 shows that rats receiving distilled water (negative control group) as treatment presented an important gain in body mass directly linked to the increase in food intake. In fact, high food intake could be due to high-fat diet given to them that increased their energy intake and energy storage (Onyeike, 2012). The gain in body mass could be explained either by the accumulation of fat or the increase of the number of cells containing fat (Myers et al., 2008; Padwal et al., 2016). Increase in body mass has been obtained in several studies (Bitam et al., 2004; Hamlat et al., 2008). Similarly, the results in table 2 shows an increase in cholesterol and triglyceride in rats treated with water and fed a hyperlipidaemic diet. Indeed, consumption of a high fat diet leads to a decrease of β-oxidation and an increase in the synthesis of cholesterol and triglyceride (Ashfaq et al., 2017; Verma, 2017). In figure 1 the rats of groups receiving AECT at different doses followed by the HFD and the rats of group VI receiving atorvastatin followed by HFD did not present the same gain in mass. Meanwhile we observed in these groups important significant increase in elimination of faecal lipids. However, in untreated rats, HFD resulted in a decrease in faecal lipids and an increase in body mass. It is therefore clear that administration of EACT followed by the hyperlipidic diet inhibited mass gain probably by increasing faecal excretion. This could be explained by the acceleration of the intestinal transit (Edoardo, 2017) due to the presence of dietary fiber in EACT. Dietary fiber refers to a vast range of biophysically and biochemically divergent compounds, carbohydrate polymers found in vegetables, and impacting on various physiological parameters (Brownlee, 2009; Edoardo, 2017). Faecal excretion could also be explained by the inhibitory effect of EACT on cholesterol and triglyceride metabolism by inhibiting lipase and intestinal lipid absorption. In fact, the binding of dietary fiber with phenolic compounds, bile salts, mineral ions, and digestive enzymes contribute to the lowering of cholesterol and the elimination of bile salts in the faeces (Gunness et al., 2010a; Edoardo, 2017). In other hand, dietary fiber makes bile acid to be unavailable as surfactants in the small intestine thus disturbing lipid emulsification, formation of mixed micelles, and the complete digestion of lipids and their absorption (Edoardo, 2017). Several studies established the ability of dietary fiber to prevent mass gain, mainly through satiety or fullness regulation (Faribanks et al., 2010; Mozaffarian et al., 2011; Shay et al., 2012; Clark Slavin, 2013; Li et al., 2014). Its action would be through the complexation of cholesterol derivatives from bile, and the diversion of their reabsorption to secretion out of the intestinal lumen (Corring et al., 1979; Ting Liu et al., 2016). Under normal conditions, hepatic cholesterol is converted to bile acids (cholic and chenodeoxycholic) and then to bile salts (glycocholate, taurocholate and taurochenate). These salts, bile acids and cholesterol are excreted in the bile to the intestine. Bile acids are deconjugated in the intestine by the action of bacteria, and the bile acids are reabsorbed into the ileum and transported via the portal vein to the liver. All bile acids reabsorbed by the liver are reconjugated and excreted again in the bile. This metabolic pathway is the extrahepatic bile salt cycle. All bile acids and cholesterol are highly absorbed, but a small portion
is excreted in the faeces. It is possible that AECT extract interferes with this cycle and increases the level of cholesterol and bile acids in the faeces, but this remains to be studied. Nevertheless, a reduction in cholesterol, triglyceride and LDL production was observed in animals treated with different doses of the extract. The biocompounds present in the aqueous extract of *C. thomsoniae* (Deutchoua et al., 2020a) would have interacted to disrupt or inhibit the different metabolisms. Specifically, studies have shown that dietary fiber has the capacity to lower the risk of developing cardiovascular diseases (Ning et al., 2012; Threapleton et al., 2013), and type-2 diabetes (Cho et al., 2013; Yao et al., 2014).

The increase in food intake in rats is thought to be due to a stimulation of appetite in the animals. Previous studies have demonstrated the ability of the aqueous extract of *C. thomsoniae* leaves to stimulate appetite in rats (Ngounou et al., 2021). However, further studies on the effect of the extract on ghrelin secretion remain to be elucidated.

This study showed the efficacy of aqueous extracts of *C. thomsoniae* in limiting the increase of blood cholesterol in a dose-dependent manner. Similar results were also shown on leaf extracts of a plant of the same genus *Cassia occidentalis* (Ntchapda et al., 2017). Cassia leaves in general have been reported to be rich in flavonoids, phenolic compounds, tannins and saponins (Geetha, 2017). Lowering serum cholesterol is widely reported to be an effective action in reducing the incidence of cardiovascular disease (Law et al., 2003).

Previous study showed the ability of AECT to lowering cholesterol (Deutchoua et al., 2020a) effect which could have a protective effect on the cardiovascular system. Our work showed that the EACT extract prevented the increase of total LDL and VLDL cholesterol and triglycerides in rats on a hyperlipidemic diet, and the decrease of HDL levels. Polyphenols have the ability to increase HDL-C content and lower oxidised LDL content (Covas et al., 2006). The effects of the extract were found to be dose-dependent, and close to the action of atorvastatin. In addition, some studies have illustrated that saponins have an anti-obesity property (Han et al., 2000; Zhang et al., 2014). Similarly, polyphenols are known to have a beneficial effect on cholesterol reduction (Juźwiak et al., 2005; Andreadou et al., 2006). The antihypercholesterolemic effect of saponins has been proven and could be explained by their abilities to inhibit acyl-CoA cholesterol acyl transferase (ACAT) activity (Zhao et al., 2008), and also due to the inhibitory effect of saponins on cholesterol absorption (Harwood et al., 1993). Phytochemical tests revealed the presence of polyphenol and saponins in our extract. These data are proof of the antihyperlipidemic effect of the extract which would have acted either like atorvastatin (reference drug) by inhibiting the activity of HMG-CoA reductase (key enzyme in cholesterol synthesis), or at the level of the intestinal lumen by inhibiting the reabsorption or esterification of cholesterol and the formation of triglycerides (Gotto et al., 1986) or by inhibiting acyl-CoA cholesterol acyl transferase activity (Zhao et al., 2008; Sadia et al., 2010; Waheeb et al., 2011). The aqueous extract of the leaves of *C. thomsoniae* leaves contributed by multiple mechanisms to reduce or protect the organisms against hyperlipidaemia, and consequently reduce the risk of atherogenicity, including atherosclerosis and cardiovascular diseases. Increased HDL levels are one of the mechanisms by which lipids are removed from the body (Barter, 2005). HDL have the role of removing cholesterol from tissues (they bring cholesterol from peripheral tissues to the liver), and they have antithrombolic and pro-fibrinolytic properties (Miller and Miller, 1975a; Miller and Miller, 1975b). One of the anti-atherosclerosis properties of HDL includes inhibition of endothelial adhesion molecule expression and LDL oxidation, as well as promotion of reverse cholesterol transport (Sanossian et al., 2007). Similarly, HDL may also reduce thrombotic risk through inhibition of platelet activation and aggregation (Shah et al., 2001) and may improve endothelial function by stimulating the release of prostacyclin, a hormone that inhibits platelet aggregation and has a vasodilatory action (Vinals et al., 1997).

To clearly understand the mechanism of action of AECT more studies need to be conducted: the effect on the release of prostacyclin, the secretion of ghrelin, on HMG coenzyme A reductase, the type of dietary fiber present in *C. thomsoniae* leaves.

**Conclusion**

These results obtained from the present study indicate that the aqueous extract of *C. thomsoniae* has significant antihyperlipidaemic activities by the ability to prevent the onset of hyperlipidaemia. The effects observed could probably be due to the bioactive substances it contains. However, further studies are in progress for the elucidation of the actual mechanism of action of AECT at the molecular level.

**Acknowledgements**

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**Conflict of interest**

The authors report no conflicts of interest.
Ethical approval
Ethics approval was obtained from the Ethics committee of the University of Ngaoundere.

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