International Journal of Modern Science and Technology, 2020;5(6):149-156.
ISSN: 2456-0235.
www.ijmst.co

Research Article

Effect of Methanolic Extract of *Chromolaena odorata* Leaves on Lipid Profile, Hmgco-A Reductase Enzyme Activity and haematological Activity of P407 induced Hyperlipidemic Albino Rats

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Abstract

The research was aimed at determining the effect of *Chromolaena odorata* on lipid profile, HMGCO-A reductase enzyme activity and Haematogical indices of P407 induced hyperlipidemic albino rats. The Lipid profile, HMGCO-A enzymes activity and Haematological indices of rat blood were measured. It was observed that after induction with poloxamer 407, there was an increase in the lipid profile activity from 133.30±33 to 154.23±4.55, 104.10±1.23 to 120.23±3.45. An induction with poloxamer 407 led to an increase in the HMG COA enzyme activity from 1.33±0.4 to 3.41±0.50 also there was an increase in the WBC count from 12.46±0.23 to 18.17±1.23 while there was a reduction in PCR count from 23.42±0.45 to 15.2±0.01. The values were statistically significant different down the groups. The findings of the present study demonstrated that poloxamer407 induced hyperlipidemia is associated with a change in Lipid profile, HMG-COA reductase enzyme activity and Haematological parameters which were ameliorated by treatment with *C. odorata.* The effect observed could be as a result of interactions between supplements biological active constituents and endogenous antioxidant system in the experimental animal model.

Keywords: *Chromolaena odorata*; Poloxamer 407; Methanolic extract; Hyperlidemia; HMGCO-A reductase; Haematological parameters.

Introduction

The use of plant materials for medicinal purposes is an ancient practice which has become even more relevant in modern perspective. Cost, availability, accessibility and effectiveness are some reasons attributable to the widespread use of these medicinal plants. No wonder [1] testified that active ingredients in plants exert definite pharmacological effects in the body, since the results of such investigations would most often serve as a lead for the biological evaluation of these plants and to new drug discovery.

It is well known that hypercholesterolaemia is a risk factor for cardiovascular diseases (CVD) such as atherosclerosis [2]. Several factors, including life style, a diet high in saturated fat and cholesterol, age and hypertension, have been reported to cause heart failure [3]. High cholesterol levels, in particular low-density lipoprotein cholesterol (LDL-C) levels, are mainly responsible for hypercholesterolaemia [4]. The World Health Organization (WHO) has predicted that heart diseases and stroke are becoming more deadly, with a projected combined death toll of 24 million by 2030 [5-8].

Chromolaena odorata is one of such plants that are being investigated for diverse health benefits; it is commonly called Awolowo in Igbo, Akintola in Yoruba and Obiarakara in Hausa. *Chromolaena odorata* is a rapidly growing perennial herb, a multi-stemmed shrub up to 2.5m tall in open areas. It has soft stems but the base of the shrub is woody. In shady areas, it becomes etiolated and behaves as a creeper, growing on other vegetation.

In Nigeria and most developing countries of the world, rural and urban dwellers, literate and illiterate rely heavily on herbal preparations for the treatment of various diseases [9]. The leaf of *Chromolaena ordorata* is popular as a natural remedy against diabetes, anaemia and other diseases. Recent claims have it that the plant is

Received: 06.04.2020; Received after Revision: 15.05.2020; Accepted: 17.05.2020; Published: 13.06.2020

not safe for use and that it could be toxic to organs in the body as well as it is used locally to treat hypertension, headache and diarrhea. The work seeks to prove above the concerns that it is being over used without regards for its possible adverse side effects.

Materials and methods

Materials

The chemicals used were Lipid profile kit (LDL), Lipid profile kit (HDL), Lipid profile kit (Cholesterol), Enzymatic assay kit for liver enzymes (AST), Enzymatic assay kit for liver enzymes (ALT), Enzymatic assay kit for liver enzymes (ALP), Hexane, Distilled water, Chloroform, Ethyl acetate and Methanol . All test kits was supplied by ChemuxBioScience, Inc South San Francisco, USA and of analytical grade.

The leaves of *Chromolaena ordoarata* were harvested using a sharp knife with hands properly protected with gloves in order to avoid the milky latex that exudes from the plant, which is capable of causing irritation and itching when in contact with the skin. The collection was done by a farmside in Makurdi, Benue State. The harvested leaves was air-dried at room temperature for two days then further dried in an oven at 40°C for 24 hr at the Biochemistry department

Forty (40) albino rats (male only) which were weighing between 200 – 300g before the experiment were bought from the animal farm of the college of health science, Benue State University, Makurdi and were acclimatized in the animal house college of Veterinary Medicine, University of Agriculture, Makurdi for 2 weeks while allowing them free access to standard feeds (Pfizer feed PLC, Lagos, Nigeria), and allowed water ad libitum [10].

Methodology

The extract was prepared according to methods described by [11]. The crispy leaves were ground into fine powder and preserved in moisture-free, airtight laboratory containers for further use. The powered plant material (100 g) was be macerated with N-Hexane ethylacetate and methanol each (1000 ml) in ratio of 1:10 and was agitated intermittently for 48 hr and filtered into a clean glass jar. The extract was evaporated to complete dryness in a stream of air (Fan) [12].

The most potent extract was subjected to column chromatograph to separate the extract into its component fractions. Silica gel was used in packing the column while different solvent combinations based on increasing polarity was used as the mobile phase [13]. The glass column (70 cm/ 30 cm) was packed as thus: A ball of cotton wool was put into a glass column using a metal wire. A slurry of silica gel was prepared using (60 g) of silica gel with a solvent system (Hexane/ Ethyl acetate) in the ratio of 9.5:0.5 and introduced into the column in one smooth [14,15].

The mean lethal dose (LD50) of the plant extract was conducted in order to determine a suitable dose for the evaluation of the effects of the extract using the method proposed by [16]. The animals were grouped as below:

NC. Negative control: The rats were not induced

HYPER C. Positive control: The rats were induced with Poloxamer 407 without treatment

HYPER+SD. The rats was induced with Poloxamer 407 and treated with a standard drug (Atorvastatin 20 mg/kg) BW

HYPER+C. The rats were induced with Poloxamer 407 and treated with the extract of *Chromolaena ordorata* (200 mg/kg) BW

HYPER+F1. The rats were induced with Poloxamer 407 and treated with the purified extract 1 (200 mg/kg) BW

HYPER+F2. The rats were induced with Poloxamer 407 and treated with the purified extract 2 (200 mg/kg) BW

HYPER+F3. The rats were induced with Poloxamer 407 and treated with the purified extract 3 (200 mg/kg) BW

HYPER+F4. The rats were induced with Poloxamer 407 and treated with the purified extract 4 (200 mg/kg) BW

Induction: The animals were made hypercholesterolaemic by an intraperitoneal injection of 500 mg/kg of P 407, followed by 6 hr of fasting. The P-407 solution was prepared for injection by combining the agent with saline, which was followed by refrigeration overnight to facilitate dissolution of the P-407 via the cold method [17]

The rats were treated with *Chromolaena ordorata* once daily for 14 days ad libitum. The rats were starved for 24 hr prior to the sacrifice. The blood samples were stored in a plain bottle (i.e. without anticoagulant) for the hormonal assay.

Determination of TG

TG levels was determined according to a method by [18]. About 10µl of distilled water, triglyceride standard and serum were pipetted into tubes labelled blank, standard and sample respectively. And 1000µl of reagent was pipetted into each of the tubes which was mixed and incubated at 37 ºC for 5 min. The absorbance of sample (Asample) and standard (Astandard) was measured against the reagent blank within 60 min at 500 nm wavelength.

Triglyceride concentration (mmol/l) =

$$\frac{\text{Asample x CStandard (2.19mmol/l)}}{\text{Astandard}} \quad (1)$$

Determination of LDL

The method of [19] was adopted. This is a combination of polyvinyl sulphate precipitation and enzymatic method. About 0.2 ml of subjects' sample was pipetted into the respective tubes and 0.2ml of reagent (A) pipetted into each of the tubes. The tubes were thoroughly be mixed and allowed to stand for 15 min at room temperature. Centrifugation will be done at 4000 rpm for 15 min and the supernatant was then carefully collected. About 20 µl of distilled water, cholesterol standard and sample supernatant were pipetted into the tubes labeled reagent blank, standard and sample respectively and 1.0ml of reagent (A) and pipetted into each of the tubes. The tubes were thoroughly be mixed and incubated for 10 min at 37^{o}C. The absorbance of the standard and sample were measured at 500 nm against the blank.

The cholesterol concentration in the supernatant (CSupernatant) in mmol/l was calculated using the following general formula (2).

CSupernatant = [ASample x Cstandard (5.18 mmol/l)] / AStandard (2)

The LDL cholesterol (mmol/l) in the sample was calculated as follows.

LDL cholesterol concentration = Total cholesterol – cholesterol in supernatant

Determination of HDL

The estimation of HDL was performed using the method as described by Burstein [20]. This is principally a combination of phosphotungstate precipitation and enzymatic method. About 0.2 ml of the subject's sample was pipetted into the respective tubes and 0.5ml of reagent (A) pipetted into each of the tubes. The tubes were thoroughly mixed and allowed to stand for 10 min. Centrifugation will be done at 4000 rpm for 10 min and the supernatant was then been carefully collected. About 50 µl of HDL-cholesterol standard, sample supernatant and distilled water was pipetted into the tubes labelled standard, sample and reagent blank respectively and 1.0 ml of reagent (B) pipetted into each of the tubes. The tubes was thoroughly be mixed and incubated for 10 min at 37 ºC. The absorbance of the standard and sample was measured at 500 nm against the blank.

Concentration of HDL cholesterol (mmol/l)=

$$\frac{\text{Asample x CStandard (1.36 mmol/l)}}{\text{Astandard}} \quad (3)$$

Total cholesterol was determined using the method as described by [21].

About 10µl of distilled water, cholesterol standard and serum were pipetted into tubes labeled reagent blank, standard and sample respectively and 1000 µl of reagent pipetted into each of the tubes. The tubes will be mixed and incubated for 5 min at 37^{o}C. The absorbance of the sample (Asample) and standard (Astandard) were measured against the reagent blank within 60 min at 500 nm wavelength.

TC concentration (mmol/l) =

$$\frac{\text{Asample x Cstandard (5.09 mmol/l)}}{\text{Astandard}} \quad (4)$$

Determination of HMG-CoA Reductase Enzyme Activity

The enzyme was analyzed using method described by [22]. The reaction was pre-incubated at 37ºC. Both the microsomal protein (total protein concentration 200 µg/mL) and 100 µM NADPH were added to buffer C (0.2 M KCl, 0.16 M potassium phosphate, 0.004 M EDTA, and 0.01 M dithiothreitol). The reaction was initiated with 50 µM HMG-CoA and was allowed to proceed for 60 min. When the reaction finished, the optical density at 340nm was measured using a UV1601 spectrophotometer (Shimadzu, Japan). One unit of enzyme activity was defined as the amount of NADPH consumed by 1 mg enzyme per minute. The system was validated via inhibition studies using three HMG-CoA reductase inhibitors: pravastatin, fluvastatin and rosuvastatin.

Statistical Analysis

The results were expressed as Mean±SD. One way ANOVA (L.S.D test) was used to test for significant difference between the groups and the normal control at $P \leq 0.05$. Duncan multiple test was also used to compare test for the difference between two groups using Statistical package for social sciences (SPSS).

Results and discussion

Table 1 shows the effect of *C.odorata* on the lipid profile of poloxamer 407 induced hyperlipidemic rats. After induction with poloxamer 407 an increase in the lipid profile activity from 133.30±33 to 154.23±4.55, 104.10±1.23 to 120.23±3.45 and from 92.34±3.67 to 113.34±5.67 in TG, LDL and Cholesterol level and a decrease of HDL from 123.45±3.40 to 78.23±4.56. The values were statistically significant different down the groups.

Table 1. Effects of *C. odorata* on the lipid profile of poloxamer 407 induced hyperlipidemic rats

Group	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Cholesterol (mg/dl)
NC	133.30±2.33^{a}	123.45±3.4^{b}	104.10±1.23^{a}	92.34±3.67^{a}
HYPER C	154.23±4.55^{d}	78.23±4.56^{a}	120.23±3..45^{c}	113.34±5.67^{d}
HYPER+SD 20mg/kg	130.23±5.6^{a}	120.12±12.33^{a}	110.34±12.34^{b}	94.34±6.78^{b}
HYPER+C 200mg/kg	145.23±1.23^{c}	136.34±2.45^{d}	103.12±1.34^{a}	98.45±2.34^{b}
HYPER+F1 200mg/kg	130.67±2.23^{a}	123.44±1.56^{b}	108.09±2.33^{b}	93.34±3.45^{a}
HYPER+F2 200mg/kg	140.21±1.23^{b}	134.56±1.23^{c}	109.23±0.89^{b}	99.34±2.24^{c}
HYPER+F3 200mg/kg	145.34±2.23^{c}	129.45±5.66^{c}	117.23±4.56^{c}	100.23±2.34^{c}
HYPER+F4 200mg/kg	138.24±1.45^{b}	128.93±3.45^{c}	114.35±2.34^{c}	97.23±1.24^{c}

Values indicated by different superscripts are statistically significant different down the group.
Key: NC=Negative control, HYPER C= Hyperlipidemic control (untreated), HYPER+SD= Hyperlipidemic + standard drug, HYPER+C= Hyperlipidemic + crude extract of C.odorata, HYPER+F1= Hyperlipidemic + first fraction, HYPER+F2= Hyperlipidemic + 2nd fraction of bioactive cpd, HYPER+F3= Hyperlipidemic + 3rd fraction of bioactive cpd, HYPER+F4= Hyperlipidemic + 4th fraction of bioactive cpd.

Hepatic impairment is one of the complications of hyperlipidemia and its evident by elevation of serum transaminase and alkaline phosphatases activities. Therefore, evaluation of serum enzymes biomarker will provide reliable indicator of functional integrity of the liver as well as treatment outcome in hyperlipidemia conditions [23]. Induction of the rats with P407 showed significant ($p \leq 0.05$) increase of low density lipoproteins (LDL), total cholesterol (TC) and triacylglycerol (TG) and decrease ($P \geq 0.05$) significantly of high density lipoproteins (HDL) (Table 1). The P407 may have been the cause of the increase of LDL, TC and TG; and decrease of HDL in the animals. This finding of the lipid reducing property of *Chromolaena odorata* is consistent with the report of [24] showed that blood lipid profile of albino wistar rats. Raised levels of serum total cholesterol and LDL have been reported to constitute risk factors in the development of cardiovascular diseases [25], this risk could be due to deposition of their cholesteryl esters during their transport in the blood vessels which results in the hardening and narrowing of the vessels which causes cardiovascular diseases, especially atherosclerosis. These observations in the animals may have been caused by the administered fresh leaf extract of *Chromolaena odorata*. Cholesterol is carried from other tissue of the body to the liver by a mediated action of high density lipoprotein (HDL) to be eliminated and low density lipoprotein (LDL) transport cholesterol to the tissues, increasing blood cholesterol.[26]reported that the medicinal plant contains saponins. Thus, the cholesterol lowering effect may have been due to the phytochemical contents, since these phytochemicals have hypocholesterolemic properties.

Table 2 shows the effect of *C.odorata* on HMG Co-A reductase enzyme activity of poloxamer 407 induced hyperlipidemic rats. An

induction with poloxamer 407 led to an increase in the enzyme activity from 1.33±0.4 to 3.41±0.50. The values were statistically significant different down the groups.

Table 2. Effects of *C. odorata* on HMG CoA reductase enzyme activity of poloxamer 407 induced hyperlipidemic rats

Group	HMG-COA reductase (mg/Kg)
NC	1.33±0.40[a]
HYPER C	3.41±0.50[b]
HYPER+SD 20mg/kg	1.20±0.18[a]
HYPER+C 200mg/kg	1.33±0.91[a]
HYPER+F1 200mg/kg	1.23±0.61[a]
HYPER+F2 200mg/kg	1.11±0.78[a]
HYPER+F3 200mg/kg	1.08±0.11[a]
HYPER+F4 200mg/kg	0.98±0.78[a]

Values indicated by different superscripts are statistically significant different down the group.
Key: NC=Negative control, HYPER C= Hyperlipidemic control (untreated), HYPER+SD= Hyperlipidemic + standard drug, HYPER+C= Hyperlipidemic + crude extract of C.odorata, HYPER+F1= Hyperlipidemic + first fraction, HYPER+F2= Hyperlipidemic + 2nd fraction of bioactive cpd, HYPER+F3= Hyperlipidemic + 3rd fraction of bioactive cpd, HYPER+F4= Hyperlipidemic + 4th fraction of bioactive cpd.

Results got from the experiment demonstrated that injection of P-407 in rats affects the enzymatic activity of hepatic microsomal HMG-CoA reductase. The effect of the surfactant on the enzymatic activity of HMG-CoA reductase appeared to be mediated by an indirect pathway; results show a significant reduction in the activity of the enzyme when incubated with P-407. Workdone by [30] suggest that the reduction in hepatic may have contributed to the specific event(s) that released the inhibition of HMG-CoA reductase synthesis. Our results indicated there was a significant reduction in HMG-CoA reductase level after treatment with both doses of ginger extract. It also implies that the extract may help manage the dyslipidemic conditions, which according to [27,28] company the administration of thiazide diuretics. Also containing statin inhibit endogenous cholesterol production by competitive inhibition of the enzyme HMG-COA reductase [29].

Table 3 shows the effect of *C.odorata* on the haematological indices of poloxamer 407 induced hyperlipidemic rats. There was an increase in the WBC count from 12.46±0.23 to 18.17±1.23, there was a reduction in the PCR count from 23.42±0.45 to 15.2±0.01 .Changes in these parameters was observed after subsequent treatment. The values varied significantly down the groups.

Table 3. Effect of *C. ordorata* crude extract and fractions on the haematological parameters of P-407 induced hyperlipidemic albino rats

Group	Hb (g/dl)	ESR (mm/hr)	PCV %	WBC (x 10^3)
NC	10.44±1.22[a]	3.76±0.56[a]	23.42±0.45[d]	12.46±0.23[a]
HYPER C	12.23±1.45[b]	8.03±0.35[c]	15.20±0.01[b]	18.17±1.23[d]
HYPER+SD 20 mg/kg	11.35±1.67[b]	3.96±1.23[a]	11.94±0.12[a]	10.22±0.37[a]
HYPER+C 200 mg/kg	11.33±1.23[b]	3.99±1.23[a]	18.23±2.33[c]	11.34±0.34[a]
HYPER+F1 200 mg/kg	10.23±2.34[a]	4.34±0.23[a]	16.23±1.22[c]	13.57±0.45[b]
HYPER+F2 200 mg/kg	11.34±2.34[b]	5.34±0.34[b]	15.83±2.34[b]	14.94±0.98[b]
HYPER+F3 200 mg/kg	11.23±1.23[b]	4.34±0.45[a]	14.34±1.34[b]	15.56±0.75[c]
HYPER+F4 200 mg/kg	11.99±2.33[b]	4.67±1.45[b]	14.23±0.98[b]	14.37±0.24[b]

Key: NC=Negative control, HYPER C= Hyperlipidemic control (untreated), HYPER+SD= Hyperlipidemic + standard drug, HYPER+C= Hyperlipidemic + crude extract of C.odorata, HYPER+F1= Hyperlipidemic + first fraction, HYPER+F2= Hyperlipidemic + 2nd fraction of bioactive cpd, HYPER+F3= Hyperlipidemic + 3rd fraction of bioactive cpd, HYPER+F4= Hyperlipidemic + 4th fraction of bioactive cpd.

Hematological indices provide physiological information on the blood picture and the reticuloendothelial system. The result of this study showed that *C. odorata* did not negatively alter white cell indices, the crude extract significantly decreased the total white cell count in *C. odorata* treated rats. This is in contrast with similar work carried out by Yakubu et al [31]. This rise in total white cell count as a result of P407 may be due to hyper stimulation of hematopoietic regulatory elements which regulates proliferation by the drug [32]. Higher quantity of flavonoids in the extracted fractions may the reason for this observed leucocytosis as flavonoids have been reported to increase intracellular vitamin C synthesis and leukocytosis [33]. This study showed significant increase in erythrocyte count in the fractions of the extracts isolated. This is confirmed by the increase in the haematocrit packed cell volume (PCV) and hemoglobin (Hb) since PCV and Hb are functions of total red blood cell as against expected anemia due to the presence of Saponins.

Conclusions

In conclusion, the findings of the present study demonstrated that poloxamer407 induced hyperlipidemia is associated with HMG-COA reductase enzymes activity and change in haematological parameters which was ameliorated by treatment with *C. odorata*. The effect observed could be as a result of interactions between supplements biological active constituents and endogenous antioxidant system in the experimental animal model.

Conflict of interest

The authors declare that there is no competing interest.

Acknowledgement

The authors wish to acknowledge the Management of Biochemistry Department, Federal University of Agriculture Makurdi, Benue State, Nigeria, for granting the authors permission to embark on the project. The authors also appreciate Mr. Ogli Itolo for his contribution towards the research.

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