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# Effect of the Aqueous Extracts of *Grewiavenusta* Leaves on Poloxamer 407-Induced Hyperlipidemic Rats

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**Abstract:** ***Hyperlipidemia is considered to be one of the greatest risk contributing to the prevalence and severity of cardiovascular diseases. The present study was taken to evaluate the effect of the aqueous leaf extract of Grewiavenusta on poloxamer 407-induced hyperlipidemic rats. The poloxamer 407 (P407) was administered using a single dose of 500mg/kg body weight which induced hyperlipidemia after 24 hours.Oral administration of extract, at dose of 200mg/kg body weight in rats, inhibited serum total cholesterol, triacyglycerides, low density lipoprotein, very low density lipoprotein and increased high density lipoprotein levels as compared to the Group IV treated with the standard drug (atorvastatin).The Results were expressed as mean±SEM by the statistical analysis-SPSS application 20.0 i.e one way ANOVA. The mean±SEM of measured hyperlipidemia induced by poloxamer 407 as 198.0±24.2 mg/dl for TAG, 247.4±30.3 mg/dl for TC. The plant aqueous extract decrease total cholesterol by 44.75% (p<0.05), triacyglyceride by 30.96% (p<0.05), VLDL by 31.06% (p<0.05), LDLc by 50.61% (p<0.05). On the other hand atorvastatin decreased total cholesterol by 33.99% (p<0.05), triacyglyceride by 35.15% (p<0.05), VLDL by 35.10% (p<0.05), LDLc by 37.42% (p<0.05).Both the plant aqueous extract and the standard drug increase the levels of HDLc significantly.***

**Keywords:** Hyperlipidemia, *Grewiavenusta*, Cardiovascular disease

## 1. Introduction

A medicinal plant is any plant in which one or more of its organs, contain substances that can be used for therapeutic purposesor which are precursors for synthesis of useful drugs [19]. It has now been established that any plant which naturally synthesizes and accumulates some secondary metabolites such as alkaloids, glycosides, tannins, volatile oils, phenols and contains minerals and vitamins possesses medicinal properties [16].The early man knew that some plant extracts could treat diseases. Galen said that there was no disease, which plants could not cure [11]. Primitive man probably found the therapeutic property of herbs by trial and error or by accident. The knowledge so obtained has been carried from generation to generation by oral tradition with almost no written form of record. However, these practices have produced results of proven efficacies [4]. In recent times herbal medicines have become indispensable and are forming an integral part of theprimary health care system of many nations [6].*Grewiavenusta*, is a specie native to north central Nigeria, most especially Middle Belt, Benue State and is distributed sparsely among the following Local Government Areas in the state; Buruku, Vandeikya, Kwande, KatsinaAla, Okpoku, Ohimini, well-rupted for its diverse medicinal uses. Different parts of this plant possess different pharmacological properties[13].*Grewiavenusta*is used by traditional herbalist in the treatment and management of fever, boils, wounds, ease of labour and it is anti-diarrhoea. Information is scanty on the use of *Grewiavenusta*as medicinal plant in the treatment and management of cardiovascular diseases. Cardiovascular disease (CVD) includes heart diseases (i.e. myocardial infarction and angina), stroke, hypertension, congestive heart failure (CHF), hardening of the arteries (anthroma, atherosclerosis) and other circulatory system diseases. Hyperlipidemia is characterized by elevated levels of serum total cholesterol (TC), low density lipoprotein (LDL-C), very low density lipoprotein (VLDL-C) and decreased high density lipoprotein (HDL-C). Such elevated serum levels of triglycerides, cholesterol, LDL and decrease levels of HDL-C are major risk factors for the premature development of cardiovascular disease like atherosclerosis, hypertension, coronary heart disease etc. It is also important risk factor in the initiation and progression of atherosclerotic impasse [17]. Hyperlipidemia associated lipid disorders are considered to cause atherosclerotic cardiovascular disease [15]. Among these hypercholesterolemia and hypertriglyceridemia are closely related to ischemic heart disease [10]. The main aim of treatment in patients with hyperlipidemia is to reduce the risk of developing ischemic heart disease or the occurrence of further cardiovascular or cerebrovascular disease [18].Atheroscerosis (Sclrero-hardening) of arteries is a generalized disease of arterial network known as a progressive and silent killer disease characterized by the formation of lesions called atherosclerosis plagues in the walls of large and or medium sized coronary arteries and reduces blood flow to the myocardium called coronary artery disease (CAD) [12]. Hyperlipidemia is classified into primary and secondary type, which indicates the complexities associated with the disease. The primary disease may be treated using anti-lipidemic drugs but the secondary type originating from diabetes, renal lipid nephrosisorhypothyroidism demands the treatment of the original disease rather than hyperlipidemia [21]. Medicinal plants play a major role in hypolipidemic activity, literature suggests that the lipid lowering action is mediated through, inhibition of hepatic cholesterol biosynthesis and reduction of lipid absorption in the intestine [8]. The present research is aimed at evaluating the efficacy and safety of the plant, *Grewiavenusta* (Family: Tiliaceae) as claimed by the traditional herbalist

in the treatment and management of cardiovascular diseases using induced hyperlipidemicwister albino rats.

## 2. Materials and Methods

### Treatment of plant part

Fresh leaves of *Grewiavenusta* was collected shade-dried and then dried in a hot air oven at 25$^{o}$C and mechanically powdered using mortar and pestle, and passed through 0.3mm mesh sieve (BS410 Endecotts Ltd London).

### Preparation of extracts

A 100g portion of the sieved sample was weighed into 1000ml conical flask and 600ml of distilled water added. It was shaken and covered with a piece of thin foil paper an allowed to stand overnight. It was shaken intermittently for maximum extraction. The aqueous extract was carefully decanted into another conical flask. Another 600ml of distilled water was added and the mixture treated the same way. The extraction was carried out for a period of seven days until the supernatant was colourless. The supernatant was then transferred into a weighed and cleaned conical flask. The extract was concentrated in the water bath at temperature of 45$\pm$ 0.5$^{o}$C until a brownish black extract was obtained. The brownish black extract obtained was ground into powder, weighed and kept for further work-$LD_{50,}$ phytochemical screening and treatment of hyperlipidemia.

### Acute toxicity of the plant ($LD_{50}$)

The acute toxicity ($LD_{50}$) test of the extract was carried out to define the range of the lethal dose and the safe range for the extract. A limit test is generally performed before the main test. One animal is dosed at the test dose i.e 2000mg/kg. If the animal dies, the main test is conducted to determine the $LD_{50}$. If the animal survives, four additional animals are dosed sequentially so that a total of five animals are tested. If three animals die, the limit test is terminated and the main test is performed. The $LD_{50}$ is less than the test dose (2000mg/kg) when three or more animals die. If a third animal dies, the main test is conducted. The $LD_{50}$ is greater than the test dose (2000mg/kg) when three or more animal survive.

If an animal unexpectedly dies late in the study, and there are other survivors, it is appropriate to stop dosing and observe all animals to see if other animals will also die during a similar observation period. Late deaths should be counted the same as other deaths (American Society of Testing and Materials, 1987).

### Phytochemical Screening of *Grewiavenusta*

Phytochemical tests were carried out with the aqueous extract of *Grewiavenusta*using standard procedure to identify the constituents [20].

### Test for tannins

0.5 ml of aqueous extract was taken and few drops of 0.1% ferric chloride was added and observed brownish green or blue black coloration.

### Test for phlobatannins

1 ml of aqueous extract was boiled with 1% aqueous hydrochloric acid deposition of red precipitate indicates the presence of phylobatannins.

### Test for saponins

10 ml of the aqueous extract was mixed with 5 ml of distilled water and shaken vigorously for stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously then observed for the formation of emulsion.

### Test of flavonoids

5 ml of dilute ammonia solution was added to aqueous filtrate extract, followed by the addition of concentrated sulphuric acid. The yellow color indicates the presence of flavonoids.

### Test for steroids

To 0.5 ml of aqueous filtrate extract of *Grewia venusta*1ml of acetic anhydride and 2ml of sulphuric acid was added. The change of color from violet to blue or green indicates the presence of steroids.

### Test for terpenoids

To 5 ml of ethanolic extract of *Grewia venusta*2 ml of chloroform and 3ml of concentrated sulphuric acid was added to form a layer. The formation of reddish brown color at the interface indicates the presence of Terpenoids.

### Test for cardiac glycosides

To 5 ml of aqueous filtrate extract of *Grewiavenusta* 2 ml of glacial acetic acid and one drop of ferric chloride solution was added followed by 1ml of concentrated sulphuric acid. The formation of brown ring indicates the presence of deoxy sugars.

### Animals

Wister albino rats weighing between 100-120g were obtained from college of Health sciences, Benue state university animal house. Animals were housed in well ventilated standard cages with day night (12 hour light dark cycle) light on at 0.7:005. Fresh, dry husk was used as bed material in the cages. The animal room was adequately ventilated and kept at room temperature and relative humidity of 29+2$^{o}$Cand good hygiene was maintained by constant cleaning and removal of faces and spilled feed from cages daily. The animals were acclimatized for 2 weeks. There were fed with commercial pellet diet (finisher, the formulation given in appendix I),

obtained from plamingo Agro feeds and foods from Gboko and they had free access from tap water.

**Experimental design**

Animals were divided into four different groups with five animals, each.

**Group I:** Normal Control Group (NC): not induced with poloxamer 407 (p407).
**Group II:** hyperlipidemia control group (HyperCp407): rats were induced using poloxamer 407 and not treated.
**Group III:** Test group A (Hyper+Aq200): rats induced with p407 and treated with *Grewia venusta* leaves extract 200mg/kg.
**Group IV:** Test group B (Hyper+Std): rats induced with p407 and treated with the standard drug (Atorvastatin).

**Cholesterol induction (poloxamer 407 induced models)**

The poloxamer 407solution was prepared by dissolving 3g in 30ml of cold normal saline. Then kept overnight in a refrigerator to facilitate its homogeneity. The syringes and needles are kept in the fridge likewise. Hyperlipidemia was induced in wister albino rats by single intraperitoneal injection of freshly prepared solution of poloxamer407 (500mg/kg) in physiological saline after overnight fasting for 18hours by a method described by [9]. The rats were divided into four groups of four rats in each group and were treated with single dose/day (p.o) of standard drug (atorvastatin) or extracts.

Group I was not induced with poloxamer 407 which serve as normal control (NC) and Group II to IV were induced with p407, (500mg/kg). Group II as hyperlipidemiccontrol/experimental control (HyperCp407) group, Group III induced with p407 and treated with plant extract 200mg/kg and Group IV induced and treated with a standard drug (atorvastation) 80mg/kg (p.o) for 4 days (served as standard).

**Assessment of biochemical parameters**

**Collection of blood**

On the final day of treatment, the animals were subjected to overnight fasting but were permitted for water. From all the groups of animal, the rats were anesthetized with chloroform and 2ml of blood sample were drawn using intracardiac puncture (plate-IV). The collected blood was centrifuged at 3000 rpm for 10 minutes. Supernatant clear serum thus obtained was transferred carefully with the help of micropipette into small test tubes and store at -20$^{o}$C prior to analysis.

**Determination of serum total cholesterol**

The principle: The enzymatic reaction sequence employed in the assay of cholesterol is as follows:

C. Esterase
Cholesterol Esters ----------► Cholesterol + fatty acid
C. Oxidase
Cholesterol + $O_2$ ----------► Cholesterol-3-one + $H_2O_2$
H. peroxidase
$2H_2O_2$+4-Aminoantipyrine+ p-HBS----------►Quinoneimine +$2H_2O$
(red dye)

Cholesterol esters are hydrolyzes to produce cholesterol. Hydrogen peroxide is then produced from the oxidation ofn cholesterol-by-cholesterol oxidase. In a coupled reaction catalyzed by peroxidise, quinoneimine dye colored red is formed from 4-aminantipyrine, p-HBS, and hydrogen peroxide. The absorption at 520nm of the solution of this dye is proportional to the concentration of cholesterol in the sample.

The routine procedures include:

Prepare reagent according to instructions on vial label. Label test tubes: blank, standard, control, rats' blood samples etc. pipette 1.0ml of reagent to all tubes and pre-warm at 37$^{o}$C for at least two (2) minutes. Add 0.01ml (10µl) of sample to respective tubes. Mix, and return to 37$^{o}$C. Incubate all tubes at 37$^{o}$C for ten (10) minutes. Zero spectrophotometer with the reagent blank at 520nm. Read and record absorbance of all tubes [22].

**Determination of triacyglyceride (TAG)**

The principle of the biochemical analysis is thus:

The enzymatic reaction sequence employed in the assay of TAG is as follows:

Lipase
TAG ----------► Glycerol + fatty acids
Glycerol kinase

Glycerol + ATP ----------► Glycerol-1-phosphate + ADP
GIP Oxidase
Glycerol-1-phosphate + $O_2$ ----------► DAP + $H_2O_2$
Peroxidase
$H_2O_2$ + 4AAP + DHBS----------► Quinoneimine Dye + $2H_2O$

The present procedure involves hydrolysis of TAG by lipase. The glycerol concentration is the determined by enzymatic assay coupled with Trinder reaction that terminates in the formation of a quinoneimine dye. The amount of the dye formed, determined by its absorption at 520nm, is directly proportional to the concentration of TAG in the samples. The routine procedure includes:

Prepare reagent according to instructions on vial label. Label test tubes: blank, standard, control, rats' blood samples etc. pipette 1.0ml of reagent to all tubes and pre-warm at 37$^o$C. Place all tubes in a 37$^o$C. Heating block for at least 4 minutes. Add 0.01ml (10µl) of sample to respective tubes. Mix, and return to 37$^o$C incubate all tubes at 37$^0$Cfor five (5) minutes. Zero spectrophotometer with the reagent blank at 520nm. Read and record absorbance of all tubes [22].

**Determination of direct HDL cholesterol (HDLc)**

The principle:

The direct HDL Cholesterol assay is a homogenous method for directly measuring serum HDLc levels without the need for any off-line pretreatment or centrifugation steps. The method is in a two-reagent format. The first reagent stabilizes LDL, VLDL and chylomicrons. The second reagent contains PEG modified enzymes that selectively react with the cholesterol present in the HDL particles. Consequently, only the HDLc is subject to cholesterol measurement.

The routine procedures include:

Use 4µl sample with 300µl of direct HDL Cholesterol Reagent 1. Equilibrate to 37$^0$Cfor 5 minutes. Add 100µl of Direct HDL Cholesterol Reagent 2. Equilibrate to 37$^0$Cfor 5 minutes measurement (Absorbance Difference between 700nm & 600nm).

**Determination of VLDL and LDL cholesterol**

The low density lipoprotein cholesterol (LDLc) and very low density lipoprotein cholesterol (VLDL) were calculated using the following formulae [7].

LDL= TC – (HDL-C +TG/5)
VLDL=TG/5

**Statistical analysis**

All values are expressed as mean±S.E.M; multiple comparisons were performed with a one-way Analysis of Variance (ANOVA) followed by Dunnett's test to determine the difference between the group means. Value was considered significant for $P<0.05$.

## 3. Results and Discussion

A number of clinical studies suggest that the increased risk of coronary heart disease is associated with a high serum concentration of TC, LDL-c, and triacyglyceride. The abnormal high concentration of serum lipids is mainly due to the increase in the mobilization of free fatty acids from the peripheral depots [1]. On the other hand, low serum concentration of HDL-c is also responsible for coronary heart disease [14]. Preclinical observations demonstrate that hyperlipidemia promotes accumulation of oxidatively modified low density lipoprotein (OX-LDL) in the arterial wall, promoting endothelial dysfunction and development of atherosclerosis and congestive heart diseases [2], [22].The physiological effect of flavanoids include possible antioxidant activity, therefore, suggestion their role in prevention of coronary heart disease including atherosclerosis [5]. Flavanoids may also work by making liver cells more efficient to remove LDL-c from blood by increasing the LDL-c receptor densities in liver and by binding to apolipoprotein B [3].Chemical studies on *Grewiavenusta* have reported the presence of Tannins, Saponins, Flavanoids and Terpenoids as main chemicals constituents and were confirmed by phytochemical screening. One or more of these pharmacology active compounds is/are likely to have contributed for the observed hypolipidemic activity of *Grewiavenusta*. From the results presented in table 2 the antihyperlipidemic activity of *Grewiavenusta* reduced cholesterol level more than the standard drug (atorvastatin) and triacyglyceride less than the standard drug (atorvastatin). In the induction of hyperlipidemia with poloxamer407, both hypertriacyglyceridemia and hypercholesterolemia (150-199mg/dl borderline high) was achieved. Group II and IV that hypertriacyglyceridemia and hypercholesterolemia was induced underwent treatment with the aqueous extract and atorvastatin for 4 days, the TAG reduced by 30.96% compared to the standard drug (atorvastatin) 35.15%, and TC reduced by 44.75% compared to the standard drug (atorvastatin) 33.99%. The values obtained for HDLc after the treatment with standard drug (atorvastatin) and aqueous extract increase appreciably. Values for LDLc were calculated using Frieldewald equation, that is LDL=TC- (HDL-c + TG/5) (mg/dl). From the data, the LDLc shows notable decrease when treated with atorvastatin (37.42%) and aqueous extract (50.61%).The values of VLDL were obtained by calculation using Frieldewald equation, VLDL=TG/5 (mg/dl). The decreased of VLDL by atorvastatin is 35.10% while the aqueous extract is 31.06%. The reduction of VLDL can be improved by increasing the period of treatment.

**Table 1:** The results of the phytochemicals screening are tabulated below:

| Tests | Result |
|---|---|
| **Tannins** | ++ |
| **Saponins** | + |
| **Flavanoids** | + |
| **Cardial glycosides** | - |
| **Terpenoids** | + |
| **Steroids** | - |
| **Phlobatannins** | - |

**Key**
**+ = Present**
**_ = Negative**

**Table 2:** The table below is the mean ± SEM of serum biochemical changes of hyperlipidemic and non-hyperlipidemic rats treated with aqueous leaf extract of *Grewiavenusta* and atorvastatin for 4 days

| Group | Serum TAG (mg/dl) | Serum TC (mg/dl) | Serum LDLc (mg/dl) | Serum HDLc (mg/dl) | Serum VLDL (mg/dl) |
|---|---|---|---|---|---|
| NC | $100.5\pm15.6^{a}$ | $140.4\pm15.4^{b}$ | $111.3\pm12.3^{b}$ | $44.43\pm2.5^{b}$ | $20.1\pm3.1^{a}$ |
| HyperCp407 | $198.0\pm24.2^{d}$ | $247.4\pm30.3^{d}$ | $205.5\pm31.4^{d}$ | $11.60\pm1.9^{a}$ | $39.6\pm4.8^{d}$ |
| Hyper+Aq200 | $136.7\pm6.9^{c}$ | $136.7\pm6.9^{a}$ | $101.5\pm6.9^{a}$ | $41.5\pm1.8^{b}$ | $27.3\pm1.4^{c}$ |
| Hyper+Std | $128.4\pm5.2^{b}$ | $163.30\pm5.2^{c}$ | $128.6\pm5.2^{c}$ | $43.5\pm5.2^{b}$ | $25.7\pm1.0^{b}$ |

Values with different superscripts down the column are statistically significant.
**NC:** Normal control, **HyperCp407:** Hyperli[pidemic rats control, induced by poloxamer 407
**Hyper+Aq200:** Hyperlipidemic rats + aqueous extract (200mg/kg)
**Hyper+Std:** Hyperlipidemic rats + Standard drug (atorvastatin 80mg/kg)

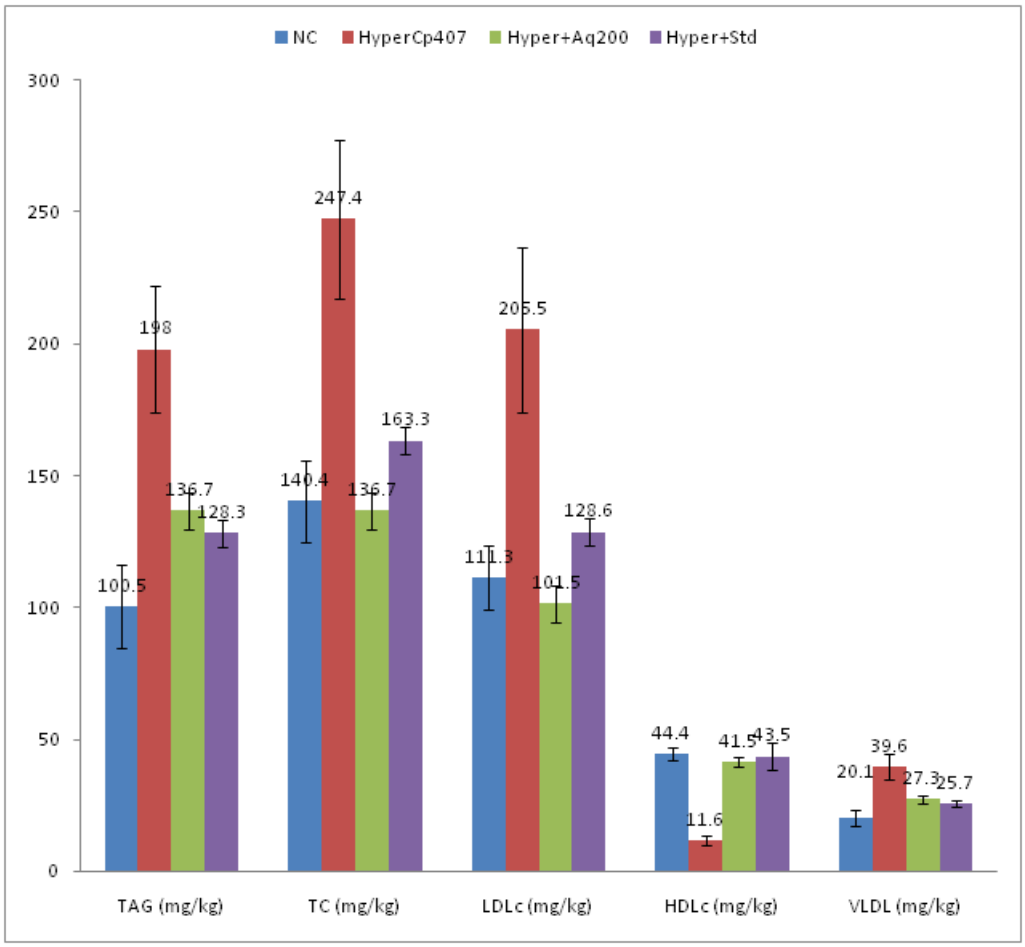


**Chart 1:** Representing data of the mean±SEM of serum biochemical changes of hyperlipidemic and non-hyperlipidemic rats treated with aqueous leaf extract of *Grewiavenusta* and atorvastatin for 4 days.

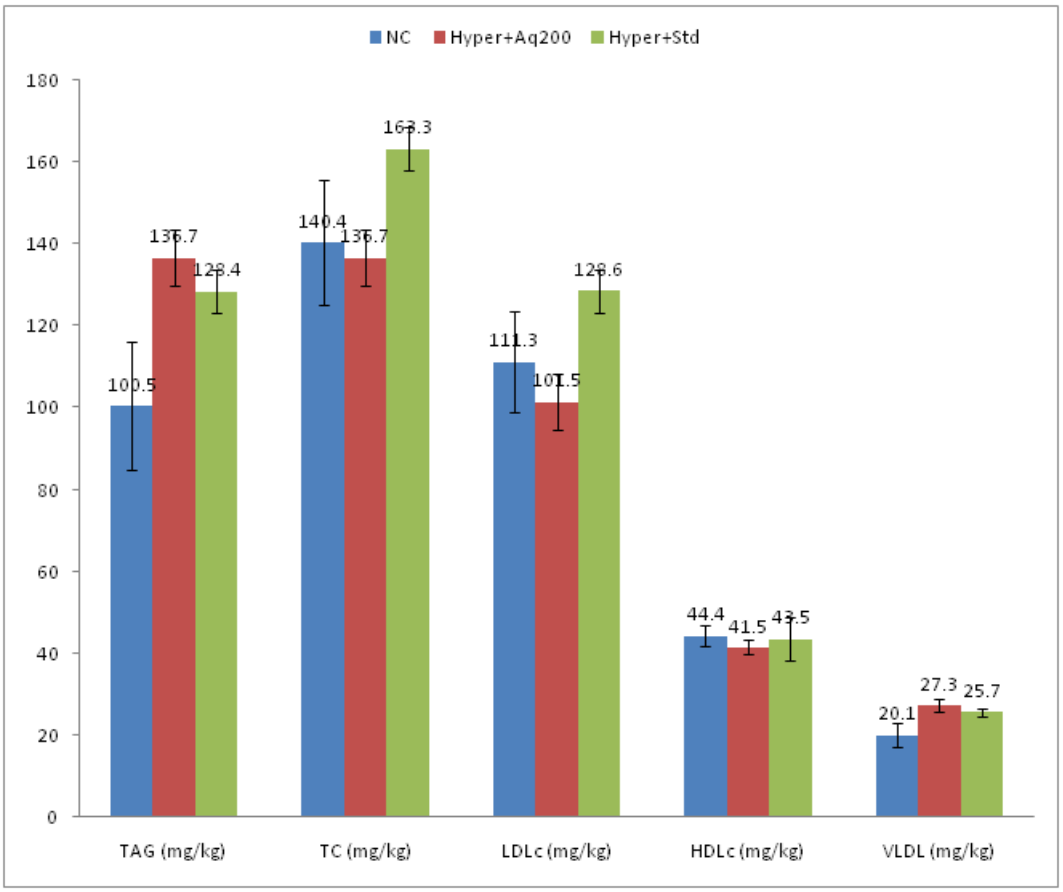


**Chart 2:** Comparing Data for NC, Hyper+ Aq200 and Hyper+Std.

## 4. Conclusion

The results of the study have shown that the administration of the aqueous leaves extracts of *Grewiavenusta* at dose level 200mg/kg is effective as hypolipidemic agent.

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