GSC Advanced Research and Reviews

eISSN: 2582-4597 CODEN (USA): GARRC2

Cross Ref DOI: 10.30574/gscarr

Journal homepage: https://gsconlinepress.com/journals/gscarr/

(RESEARCH ARTICLE)

Check for updates

Effect of leaf extract of *Terminalia catappa* on haematological profile in poloxamer induced hypercholesterolemia in Wistar rats

Dasofunjo K [1,*], Nwachukwu GC [1], Emerole CG [2], Nnamani VI [3], Asuquo UU [1], Ejim C I [4], Wilson AO [5] and Ogar B.O [1]

[1] *Department of Medical Biochemistry, University of Cross River State, Okuku, Cross River State, Nigeria.*
[2] *Optometry Unit, Eye clinic, Federal University Teaching Hospital, Owerri, Nigeria.*
[3] *Department of Biochemistry, University of Nigeria, Nsukka, Enugu State, Nigeria.*
[4] *Department of Physiology, University of Cross River State, Okuku, Cross River State Nigeria.*
[5] *Department of Medical Laboratory Science, College of Health Technology, Calabar, Cross River State, Nigeria.*

GSC Advanced Research and Reviews, 2023, 17(01), 107–116

Publication history: Received on 04 August 2023; revised on 16 October 2023; accepted on 18 October 2023

Article DOI: https://doi.org/10.30574/gscarr.2023.17.1.0364

Abstract

This research was aimed at assessing the effect of the administration of ethanol extract of *Terminalia catappa*, on haematological indices following poloxamer induced hypercholesterolemia in male Wistar rats. Thirty- five (35) Wistar male rats were allowed to acclimatize for a period of 7 days, in a well-ventilated room at room temperature and relative humidity of 29°C and 70% respectively with 12 hours natural light-dark cycle. They were allowed food and water *ad libitum*. Good hygiene was maintained by daily cleaning and removal of faeces and spills from their cages. The rats were randomly divided into 5 groups of 7 rats each. Group A: were fed with standard chow and distilled water (NC). Group B: were induced with 1.0mg/kg dose of P-407 without treatment (HC). Group C: were induced with 1.0 mg/kg dose of P-407 and treated with atorvastatin (ATV) at 20mg/kg body weight/day Group D: were induced with 1.0mg/kg dose of P-407 and treated with leaves extract (HLE) at 100mg/kg body weight/day Group E: were induced with 1.0mg/kg dose of P-407 and treated with leaf extract (HLE) at 200mg/kg body weight/ day. The dose regimens were administered once daily for 14 days. The rats were monitored for clinical signs and death. The result reveals that there was a significant ($P<0.05$) increase in RBC, Hb and WBC when compared to the normal, standard and hypercholesterolemic group in the group treated with *T. catappa* ethanol extract. Similarly, there was a significant ($P<0.05$) decrease in PCV when compared with the normal, standard and hypercholesterolemic control. On the other hand, the extract produced no significant($P<0.05$) difference in serum MCV, MCH and MCHC, neutrophil, platelet and lymphocytes when compared with the normal, standard and hypercholesterolemic control.It can be inferred from this present research work that *T.catappa* contains some phytochemical, and/or neutraceuticals with haematopoietic and immunomodulatory effect which may trigger or boost the defense system against invasion by either pathogens or pathogenic organisms in poloxamer induced hypercholesterolemic rats.

Keywords: Cardiovascular diseases; Haematopoietic; Hypercholesterolemia; Immunomodulation; Poloxamer

1. Introduction

Hypercholesterolemia is considered one of the major risk factors causing cardiovascular diseases (CVDs). CVDs accounts for one third of total deaths around the world, it is believed that CVDs will turn out to be the main cause of death and disability globally by the year 2013 [1]. Hypercholesterolemia is an increase in one or more of the plasma lipids, including triglycerides, cholesterol, cholesterol esters and phospholipids and or plasma lipoproteins including very low-density lipoprotein and low-density lipoprotein, and reduced high-density lipoprotein levels [2].

*Corresponding author: Dasofunjo K.

Several scientific reports have shown that elevated levels of LDL cholesterol increase a person's risk or predisposition to atherosclerotic plaques and subsequent vascular disease. Contrastingly, high-density lipoprotein (HDL) cholesterol assists in regulating cholesterol levels to prevent imbalances that would increase the risk of atherosclerotic vascular disease. Hypercholesterolemia and hypertriglyceridemia are the main cause of atherosclerosis which is strongly related to ischemic heart disease (IHD) [3]. There is a strong relationship between HDL and the high mortality rate. Furthermore, elevated plasma cholesterol levels cause more than four million deaths in a year [4]. Hypercholesterolemia can be subdivided into two broad classifications: primary (familial) or secondary (acquired) hyperlipidemia. Primary hypercholesterolemia derives from a plethora of genetic disorders that a patient may inherit through birth, while secondary hypercholesterolemia typically originates from an alternate underlying etiology, such as an unhealthy diet, medications (amiodarone, glucocorticoids), hypothyroidism, uncontrolled diabetes, and/or a poor lifestyle regimen [5].

Hypercholesterolemia is typically a chronic, progressive disease process that demands lifestyle and dietary changes, with the potential need for additional lipid-lowering medications. The degree of hypercholesterolemia is highest in patients with premature coronary artery disease (CAD), defined as CAD arising in males before age 55 to 60 years and females before age 65 years. Under the prior specified circumstances, the incidence of hypercholesterolemia is around 75-85%, opposed to roughly 40 to 48% in the control population of comparable age, but without the presence of premature coronary artery disease [6].

Regularly, patients presenting with underlying hypercholesterolemia remain asymptomatic, therefore obtaining a precise and thorough history is essential. Upon taking a patients history, it is crucial to obtain a profound understanding of each patient's family history of cardiovascular disease, hyperlipidemia, and/or familial hypercholesterolemia, their diet and exercise habits, tobacco, alcohol, or drug use, the presence of coronary artery disease, risk factors or history of CAD, and/or symptoms of peripheral arterial disease or angina [7].

In addition to obtaining a detailed history, a focused physical examination is also very important. Obtaining accurate blood pressure measurements, observing the patients skin for xanthomas, listening for carotid and femoral bruits for evidence of stenosis, listening for an S4 heart sound, and palpating for intact peripheral pulses in all four extremities are fast and simple physical examination that can assist the diagnosis of hypercholesterolemia [8].

Hypercholesterolemia is often a life-long disease process, but one that is typically quite manageable. However, if hypercholesterolemia is left untreated, the disease is progressive and will often lead to severe underlying vascular diseases, which can prove fatal. Ongoing persistent exposure to high serum lipid levels throughout early adulthood increases the person's subsequent risk of coronary heart disease in a dose-dependent manner [9].

Adults with ongoing exposure to moderate or severe elevations in non-HDL cholesterol levels have concurrent elevated risks for developing coronary heart disease and would likely benefit from aggressive medical treatment modalities which includes high-intensity statin therapy in addition to diet and lifestyle modifications [10] .

Aerobic exercise has shown to increase the levels of the HDL-cholesterol protein, with an anti-atherogenic action: the HDL that transports cholesterol from the walls of the arteries and peripheral tissues to the liver and the same HDL has an antioxidant and anti-inflammatory capacity that induces the release of nitric oxide (vasodilator compound). Estimates are that adding 10 minutes of physical activity to daily exercise increases the concentration of HDL by 1.4 mg/dl, and it has been calculated that, on average, an adequate training program can increase cholesterol HDL by 4.6% [11, 12]. A combination of aerobic and anaerobic activity is equally capable of positively influencing blood lipid levels [13].One hour per week of resistance training (anaerobic activity) can improve the lipid profile and corresponds to a correct indication in order not to exceed in physical effort and maintain optimal health [14].

Termilania catappa is a well-recognized herb in Ayurveda. The juice of its fresh leaves is used in the preparation of medicinal lotion for leprosy and scabies, and it is taken internally for stomach ache and headache, dermatitis, hepatitis, diarrhea and pyresis [15]. This plant was also listed in Pharmacopeia vegetables of the Caribbean, where the leaves of this plant are used in a decoction for gastritis and urinary infection [16]. The leaves have many medicinal uses including diaphoretic, anti-indigestion, and anti-dysentery [15]. An infusion of the young leaves or scraped bark is occasionally taken as a potion for treating mouth infections, cure headache and colic. The bark is used as an astringent in dysentery and thrush [17].This present research work was designed to determine the effect of *T. cattapa* on haematological indices following poloxamer induced hypercholesterolemia in Wistar rats.

2. Materials and Methods

2.1. Plant materials

Fresh leaves of *Terminalia catappa* was collected from UNICROSS environment, Okuku, University of Cross River State, Nigeria. The leaves were taken to the University of Calabar, Department of Botany for identification and authentication. The voucher number of 206 has been deposited for future reference at the department's herbarium.

2.2. Experimental animals

Thirty- five (35) Wistar male rats were obtained from the animal holding unit of the Department of Medical Biochemistry, University of Cross River State (UNICROSS). The animals were allowed to acclimatize for a period of 7 days, in a well-ventilated room at room temperature and relative humidity of 29°C and 70% respectively with 12 hours natural light-dark cycle. They were allowed food and water *ad libitum*. Good hygiene was maintained by daily cleaning and removal of faeces and spills from their cages.

2.3. Assay kits

All assays kits for Total cholesterol (TC), Triacylglycerol (TAG), High Density Lipoprotein (HDL) and Low Density Lipoprotein (LDL) were purchased from Randox laboratories Ltd® (Northern Ireland, UK), Ardmore, Co. Antrum UK.

2.4. Preparation of extract of *Terminalia catappa* leaf

The leaves of *Terminalia catappa* was collected around University of Cross River State (UNICROSS) and air dried at room temperature for a period of 21 days until constant weight was obtained. The dried leaves were then pulverized to powdered form by a machine blender and sieved. Thereafter, 400g of the pulverized plant material (*Terminalia catappa*) was dissolved in 1200ml of 70% ethanol for 72 hours. This was followed with vacuum filtration and extracts was concentrated using an evaporator water bath at 40°C to obtain a solvent free extract, and stored in a refrigerator at 4°C. Preparation of standard drug: Atorvastatin (Pfizer Ireland Pharmaceuticals, Ireland) was purchased in a tablet form at strength 20mg. Tablets were crushed into powder, dissolved in distilled water and administered orally.

2.5. Induction of hypercholesterolemia

Poloxamer 407 (Lutrol F127; BASF, Ludwigshafen, Germany) was used as the inducing agent. Hypercholesterolemia was induced by injecting 1.0g/kg dose of P-407 intraperitoneally. All syringes were placed on ice prior to P-407 administration to maintain the polymer in a mobile viscous state during the injection, since P407 solutions at concentrations greater than about 23% w/w exhibit reverse thermal gelatin properties.

2.6. Experimental design

A total of 35 healthy male Wistars rats were used. The rats were randomly divided into 5 groups of 7 rats each. Group A: were fed with normal chow and distilled water only (NC). Group B: were induced with 1.0 mg/kg dose of P-407 (HC). Group C: were induced with 1.0 mg/kg dose of P-407 and treated with Atorvastatin (ATV) at 20mg/kg body weight Group D: were induced with 1.0mg/kg dose of P-407 and treated with leaves extract (HLE) at 100mg/kg body weight/day , Group E: were induced with 1.0 mg/kg dose of P-407 and treated with leaf extract (HLE) at 200mg/kg body weight/ day .The dose regimens were administered for 14 days and the rats were monitored for clinical signs and death.

2.7. Collection and preparation of sera samples:

At the end of the 14-days experimental period, the anaesthesia was performed on all experimental animals. The anesthetized animals were bled by cardiac puncture. The blood samples were collected and centrifuged at a speed of 3000 rpm for 15 minutes and serum collected into EDTA sample bottles for sexual hormones haematological parameters

2.8 Determination of haematological parameters

2.7.1. Determination of blood haemoglobin

Blood haemoglobin concentration was determined using cyanmethaemaglobin method [18]. Blood sample (0.02ml) was added to 5 ml Drabkin's reagent held in a test tube and the solution was inverted several times to mix properly. The mixture of blood and Drabkin reagent was allowed to stand at room temperature for 5 minutes, and thereafter poured

into a cuvette and read at 540 nm. The haemoglobin concentration in gramme per litre was then derived from the absorbance value by matching against per-determined reference standards and calibration curves.

2.7.2. Determination of packed cell volume

Packed cell volume (also called haematocrit) was determined using configuration method as described by the method of [18].The micohaematocrit tubes were filled with blood samples up to three quarter level, via capillary action. One end of the tubes was sealed with plasticein. The tube with its blood content was placed in Hawksley Microhaematocrit centrifuge at 10,000g for 5 minutes and proportion of cells to whole column (i.e the PCV) was measured using Hawksley microhaematocrit reader.

2.7.3. Determination of erythrocyte count

Erythrocyte count (also called red blood cells, RBC) was determined using the haemocytometer method [19]. Blood sample (0.02 ml) drawn with a micropipette was added to 4 ml of erythrocyte diluting fluid held in a test tube. A drop of the diluted blood sample was used to charge the neubauer chamber before counting the erythrocyte under the microscope at x 40 objective using the tally counter. Erythrocytes in the five small squares of the middle square of the Neubauer chamber were counted. A factor of 10, 00 was used to multiply the number of cells counted in the five small squares, to get the absolute number of erythrocytes per microlitre of blood.

Mean corpuscular volume (MCV): This was determined by dividing the PCV by the RBC value determined as described above and then multiplied by a constant of 10. Values obtained were expressed in femtolitre.

$$\text{MCV (F1)} = \frac{\text{PCV (\%) x 10}}{\text{RBC (x } 10^{12}\text{/L)}}$$

Mean corpuscular haemoglobin (MCH): This was calculated by dividing the heamoglobin concentration by the RBC, already determined, and the multiplied by a factor of 10. The values were expressed in pictogram.

$$\text{MCH (pg)} = \frac{\text{HB (g/dl) x 10}}{\text{RBC (x } 10^{12}\text{/L)}}$$

Mean Corpuscular Haemoglobin concentration (MCHC): This was calculated by dividing the haemoglobin concentration by the PCV value already obtained, and multiplied by 100. The values were expressed in gramme per liter.

$$\text{MCHC (g/dl)} = \frac{\text{HB (g/dl) x 100}}{\text{PCV (\%)}}$$

Determination of total leucocyte count

Total leucocytes, also called white blood cells (WBC) were determined using the haemocytometer method [19]. The blood sample (0.02 ml) collected with a micropipette was mixed with 0.38 ml of white blood cell diluting fluid held in a test tube. A drop of the diluted blood sample was used to charge the Neubauer chamber, placed on the microscope stage. White blood cells (leucocytes) were counted in the four corner squares of the Neubauer chamber under x 40 objective using the tally counter. The number of leucocytes counted in the four corner squares was multipled by a factor of 50 to get the total number of leucocytes per microlitre of blood.

2.8. Statistical analysis

The data obtained were analysed using One Way Analysis of Variance (ANOVA) followed by post hoc test at $P<0.05$. The Statistical Package for Scientific Solutions (SPSS) Software version 20.0 was used for the analysis.

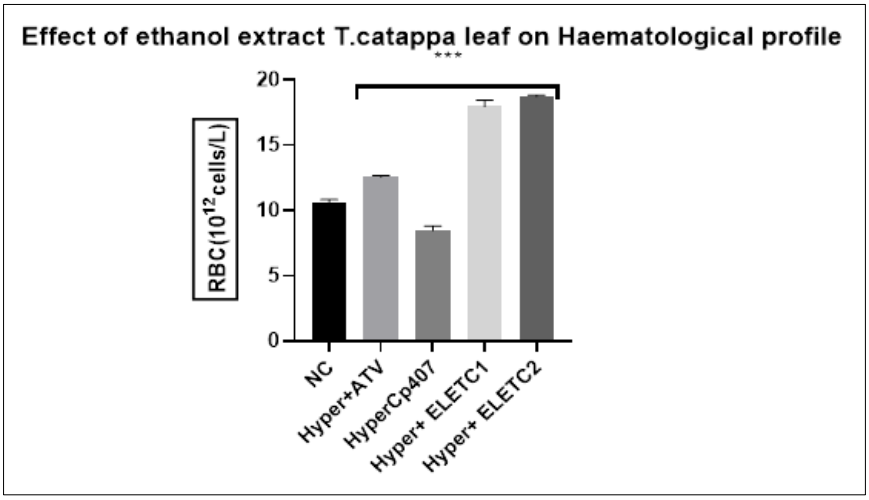
3. Result

The result below reveals the effect of ethanol extract of *T. catappa* on haematological indices following poloxamer induced hypercholesterolemia in male Wistar rat. Following the administration of the extract, the result significantly ($P<0.05$) increases RBC and Hb when compared with the normal, standard and hypercholesterolemic control (figure 1-2).

Alternatively, the extract significantly decreases the PCV when compared to the normal, standard and hypercholesterolemic control (figure 3).

More so, the extract produces no significant difference on serum MCV, MCH and MCHC when compared to the normal, standard and hypercholesterolemic control (Table 1).

Furthermore, the extract produced significant ($P<0.05$) increase in WBC following the induction of hypercholesterolemia when compared with the normal, standard and hypercholesterolemic control. The extract also produces no significant difference in neutrophil, platelet and lymphocytes when compared with the normal, standard and hypercholesterolemic control (Table 2).



* Significance

Figure 1 Effect of ethanol extract *T.catappa* leaf on RBC in poloxamer induced hypercholesterolemia in Wistar rat

Results were expressed as mean ± SD (n=5) *** significant at $P<0.05$ compared with the control. NC: Normal Control, Hyper + ATV: Hyperlipidemic rats + atorvastatin (20mg/Kgbwt) ,HyperCp407: Hyperlipidemic rats control, induced with poloxamer 407, Hyper + ELETC1: Hyperlipidemic rats + ethanol leaf extract of *Termilania catappa* (100mg/Kgbwt) a Hyper + ELETC2: Hyperlipidemic rats + ethanol leaf extract of *Termilania catappa* (200mg/Kgbwt).

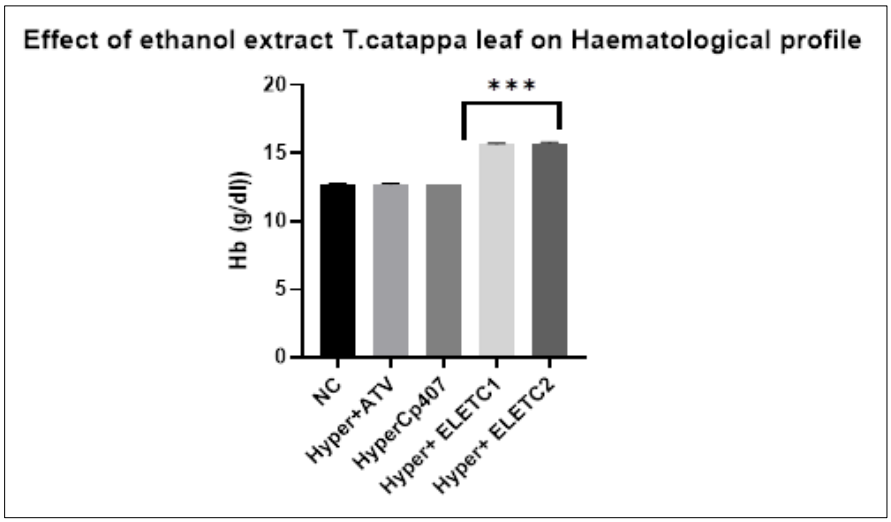


Figure 2 Effect of ethanol extract *T.catappa* leaf on Hb in poloxamer induced hypercholesterolemia in Wistar rat

Results were expressed as mean ± SD (n=5) *** significant at P<0.05 compared with the control. NC: Normal Control, Hyper + ATV: Hyperlipidemic rats + atorvastatin (20mg/Kgbwt) ,HyperCp407: Hyperlipidemic rats control, induced with poloxamer 407, Hyper + ELETC1: Hyperlipidemic rats + ethanol leaf extract of *Termilania catappa* (100mg/Kgbwt) a Hyper + ELETC2: Hyperlipidemic rats + ethanol leaf extract of *Termilania catappa* (200mg/Kgbwt).

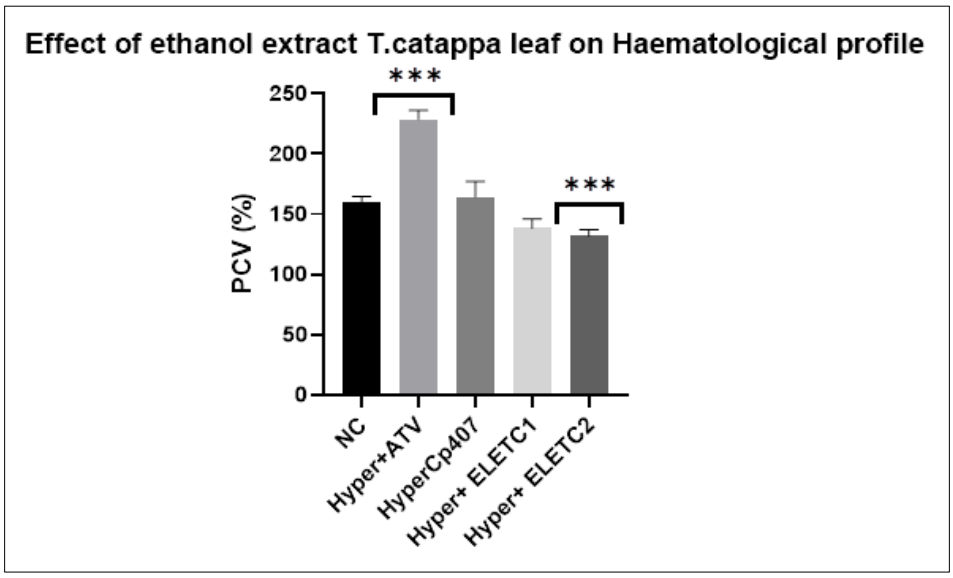


Figure 3 Effect of ethanol extract *T.catappa* leaf on PCV in poloxamer induced hypercholesterolemia in Wistar rat

Results were expressed as mean ± SD (n=5) *** significant at P<0.05 compared with the control.NC: Normal Control, Hyper + ATV: Hyperlipidemic rats + atorvastatin (20mg/Kgbwt) ,HyperCp407: Hyperlipidemic rats control, induced with poloxamer 407, Hyper + ELETC1: Hyperlipidemic rats + ethanol leaf extract of *Termilania catappa* (100mg/Kgbwt) a Hyper + ELETC2: Hyperlipidemic rats + ethanol leaf extract of *Termilania catappa* (200mg/Kgbwt).

Table 1 Effect of ethanol extract *T.catappa* leaf on MCV, MCH and MCHC in poloxamer induced hypercholestrolemia in Wistar rats

Groups	MCV (f/l)	MCH (Pg)	MCHC (g/l)
NC	51.57±0.62	38.88±0.93	728.00±21.66
Hyper + ATV	44.50±1.96*	32.83±1.05*	709.67±43.11
HyperCp407	48.97±1.85a	34.11±1.20*	706.67±16.92
Hyper + ELETC1	49.93±0.92a	35.63±0.68*	713.00±1.73
Hyper + ELETC2	52.10±1.01a	36.78±0.47a	705.33±5.84

Values are expressed as mean±SEM. (n = 5). Values with different superscript along the columns are statistically significant (P<0.05). NC: Normal Control, Hyper + ATV: Hyperlipidemic rats + atorvastatin (20mg/Kgbwt) ,HyperCp407: Hyperlipidemic rats control, induced with poloxamer 407, Hyper + ELETC1: Hyperlipidemic rats + ethanol leaf extract of *Termilania catappa* (100mg/Kgbwt) a Hyper + ELETC2: Hyperlipidemic rats + ethanol leaf extract of *Termilania catappa* (200mg/Kgbwt).

Table 2 Effect of ethanol extract *T.catappa* leaf on WBC, LYM PLT and NEU in poloxamer induced hypercholestrolemia in Wistar rats**WBC (10^9/l)**

Groups	WBC (10^9/l)	LYM (10^9/l)	PLT (10^9/l)	NEU%
NC	46.30±0.40	14.31±1.82	633.00±15.37	81.63±1.26
Hyper + ATV	68.17±0.35*	14.93±45.04	596.67±53.65	65.81±3.19*
HyperCp407	61.97±1.21*	15.46±0.63	456.00±138.69	79.41±0.71a
Hyper + ELETC1	73.03±3.52abc	15.02±0.55	729.33±40.70bc	81.69±1.07a
Hyper + ELETC2	77.00±1.32abc	13.53±0.93	756.00±3.22bc	80.20±0.53a

Values are expressed as mean±SEM. (n = 5). Values with different superscript along the columns are statistically significant (P<0.05). NC: Normal Control, Hyper + ATV: Hyperlipidemic rats + atorvastatin (20mg/Kgbwt), HyperCp407: Hyperlipidemic rats control, induced with poloxamer 407, Hyper + ELETC1: Hyperlipidemic rats + ethanol leaf extract of *Termilania catappa* (100mg/Kgbwt) a Hyper + ELETC2: Hyperlipidemic rats + ethanol leaf extract of *Termilania catappa* (200mg/Kgbwt).

4. Discussion

Atherosclerosisis a disease of the arterial wall, characterized by thickening of the intimal layer and accumulation of fat , partly caused by hyperlipidemia. Blood is a complex fluid, responsible for delivering nutrients to most body tissues and for collecting metabolic wastes, avoiding tissue accumulation and toxicity. It is composed of an extracellular fluid rich in proteins – plasma – and cells, such as erythrocytes, leukocytes and platelets [20].

Erythrocytes, or red blood cells, the main blood components, are non-nucleated cells that contain great amount of haemoglobin, a protein responsible for O_2 and CO_2 transport. Under normal conditions, erythrocytes do not leave the circulatory system: they always remain within blood vessels actively participating in homeostasis maintenance [21]. Although, their primary function is the transport of gases bound to haemoglobin, it is evident that erythrocytes have other interactions, such as the capacity of diffusional exchange of cholesterol, but with low capacity for cholesterol storage [22].Therefore, the significant increase in RBC observed from treatment with *T.cattapa* from this present study suggest the erythropoietic and or haematopoietic or haematinic effect of the extract in polyxamer induced hyperlipidaemia.

More so, white blood cell and its differentials are to fight infections, defend the body by phagocytocis against invasion by foreign organisms and to produce or at least transport and distribute antibodies in immune response. Thus, animals with low white blood cells are exposed to high risk of disease infection, while those with high counts are capable of

generating antibodies in the process of phagocytocis and have high degree of resistance to diseases [23] and enhance adaptability to local environmental and disease prevalent conditions [24]. Hence, it appears that this extract contains some phytonutrient or neutraceuticals with immunomodulatory effect which may trigger or boost the defense system against invasion by pathogens or pathogenic organisms in poloxamer induced hyperlipidaemia.

Blood platelets are implicated in blood clotting. Low platelet concentration suggests that the process of clot- formation (blood clotting) will be prolonged resulting in excessive loss of blood in the case of injury. Hypercholesterolemia stimulates platelet biogenesis through mega karyopoiesis, and leukocytosis by myelopoiesis, and increases platelet activation, by promoting platelet production and by direct impact on platelets [25]. The increased cholesterol level enhances the hyper aggregability of thrombocytes too. Activated platelets can form aggregates with neutrophils and monocytes, and the subsequent crosstalk between platelets and leukocytes also plays an important role in the production of inflammatory cytokine, in the biosynthesis of leukotrienes and reactive oxygen species (ROS) [25]. The ROS can induce the production of inflammatory mediators such as C-reactive protein (CRP), which can activate the pro-thrombotic factors and platelets [26, 27] Hence, the observed reduction in platelet following co-administration of extract *Terminalia catappa* in poloxamer induced hypercholesterolemia.

Packed Cell Volume (PCV) which is also known as haematocrit (Ht or Hct) or erythrocyte volume fraction (EVF) is the percentage of red blood cells in blood [28]. According to Peters *et al.,* [29], Packed Cell Volume, haemoglobin and mean corpuscular haemoglobin are major indices for evaluating circulatory erythrocytes, and are significant in the diagnosis of anaemia and also serve as useful indices of the bone marrow capacity to produce red blood cells as in mammals [30]. Furthermore, Chineke *et al.,* [31] posited that high Packed Cell Volume (PCV) reading indicated either an increase in number of Red Blood Cells (RBCs) or reduction in circulating plasma volume. Mean corpuscular, haemoglobin and mean corpuscular haemoglobin concentration indicate blood level conditions. The significant decrease observed in PCV following the administration of the extract also suggests its haematinic ability in hyperlipidaemic rats.

5. Conclusion

It can be inferred from this present research work that *T.catappa* contains some phytochemical, and/or neutraceuticals with haematopoietic and immunomodulatory effect which may trigger or boost the defense system against invasion by pathogens or pathogenic organisms in poloxamer induced hypercholesterolemia.

Compliance with ethical standards

Acknowledgments

The authors of this research study wish to appreciate the technical assistance of Mr Obogo Ezekiel of the Department of Medical Biochemistry, University of Cross River State, Okuku Campus, Nigeria.

Disclosure of conflict of interest

The authors have declared no conflict of interest.

Statement of ethical approval

Ethical approval for the study was obtained from the Faculty of Basic Medical Science Animal Research Ethical Committee of Cross River University of Technology, Calabar, Nigeria (approval number FBMS/UNICROSS/21/025).

References

[1] Jorgensen T, Capewell S, Prescott E, Allender S, Sans S ,Zdrojewski T . Population-level changes to promote cardiovascular health. *European Journal of Preventive Cardiology.* 2013,**20**(3):409-421.

[2] Mishra P R, Apanna KC, Panigrahi S. (2011) Evaluation of acute hypolipidemic activity of different plant extracts in Triton WR-1339 induced Hypercholesterolemia in albino rats. *Pharmacology online*. **3**:925-934.

[3] Brouwers M C,VanGreevenbroek M M, Stehouwer C D,de Graaf J, Stalenhoef A F, The genetics of familial combined hyperlipidaemia. *Nature Reviews Endocrinology.* 2012,**8**(6): 352-362.

[4] Kumar D, Parcha V,Maithani A, Dhulia I .Effect & evaluation of antihyperlipidemic activity guided isolated fraction from total methanol extract of Bauhinia variegata (linn.) in Triton WR–1339 induced hyperlipidemic rats. *Asian Pacific Journal of Tropical Diseases.* 2012,**2**(2):909-913.

[5] Ballantyne C M , Grundy S M, Oberman A, Kreisberg R A, Havel R J, Frost P H, Haffner S M. Hyperlipidemia: diagnostic & therapeutic perspectives. *The Journal of Clinical Endocrinology & Metabolism.* 2000,**85**(6):2089-2092.

[6] Fredrickson D S.An international classification of hyperlipidemias & hyperlipoproteinemias. *Annals of Internal Medicine.* 2011,**75**(3):471-472.

[7] Vodnala D, Rubenfire M ,Brook R D. Secondary causes of dyslipidemia. *American Journal of Cardiology.* 2012,**110**(6):823-825.

[8] Vallejo-Vaz A J, Robertson M, Catapano A L , Watts G F, Kastelein J J, Packard C J, Ford I, Ray K K. Low-density lipoprotein cholesterol lowering for the primary prevention of cardiovascular disease among men with primary elevations of low-density lipoprotein cholesterol levels of 190 mg/dl or above: analyses from the woscops (west of scotl& coronary prevention study) 5-year r&omized trial & 20-year observational follow-up. *Circulation.* 2017,**136**(20):1878-1891.

[9] Ford I, Murray H, McCowan C ,Packard C J. Long-term safety and efficacy of lowering low-density lipoprotein cholesterol with statin therapy: 20-year follow-up of west of scotl& coronary prevention study. *Circulation*. 2016,**133**(11):1073-1080.

[10] Ford I, Murray H, McCowan C ,Packard C J. Long-term safety and efficacy of lowering low-density lipoprotein cholesterol with statin therapy: 20-year follow-up of west of scotl& coronary prevention study. *Circulation*. 2016,**133**(11):1073-1080

[11] Fathalla Z M , Vangala A, Longman M , Khaled K A , Hussein A K , El-Garhy OH Alany RG. Poloxamer-based thermoresponsive ketorolac tromethamine in situ gel preparations: Design, characterisation, toxicity & transcorneal permeation studies. *European Journal of Pharmaceutics & Biopharmaceutics.* 2017,**114**: 119–134.

[12] Franklin B A, Brubaker P, Harber M P, Lavie C J, Myers J , Kaminsky L A. The journal of cardiopulmonary rehabilitation & prevention at 40 yr & its role in promoting preventive cardiology: part 2. *Journal of Cardiopulmonary, Rehabilitation & Prevention.* 2020, **40**(4):209-214.

[13] Álvarez C, Ramírez-Campillo R, Lucia A.Ramírez-Vélez R.. Izquierdo M. Concurrent exercise training on hyperglycemia & comorbidities associated: Non-responders using clinical cutoff points. *Sc&inavian Journal of Medicine & Science in Sports.* 2019,**29**(7):952-967.

[14] Bakker E A, Lee D C, Sui X, Eijsvogels T M H, Ortega F B, Lee I M, Lavie C J ,Blair S N,. Association of resistance exercise with the incidence of hypercholesterolemia in men. *Mayo Clinic Proceedings.* 2018, **93**(4):419-428

[15] Azrul L M, Adzemi M A, Ahmad W M , Effendy AW. Determination of toxicological effects of *Terminalia catappa* leaves on Sprague Dawley white rats in short term period. *International Journal Toxicology & Applied Pharmacology,* 2013,**3**:447-453

[16] Tomaiuolo G. Biomechanical properties of red blood cells in health & disease towards microfluidics. *Biomicrofluidics.* 2014, **8,** 051501. https://doi.org/10.1063/1.4895755

[17] Quartey GA. Mechanical Properties of *Terminalia catappa* from Ghana. Materials Sciences and Applications.2022,13, 334-341. https://doi.org/10.4236/msa.2022.135018

[18] Barale C, Cavalot F, Frascaroli C, Bonomo K, Morotti A , Guerrasio A, Russo I. Association between high on-aspirin platelet reactivity & reduced superoxide dismutase activity in patients affected by type 2 diabetes mellitus or primary hypercholesterolemia. *International Journal Molecular Science.* 2020, **21,** 4983

[19] Coles EH.Veterinary Clinical Pathology. 4th Edition, W.B. Saunders Company, Philadelphia, 1986, 17-19.

[20] Rafieian-Kopaei, M, Setorki M, Doudi M, Baradaran A,Nasri H. Atherosclerosis: Process, indicators, risk factors & new hopes. *International Journal of Preview Medicine.*2014,**5,** 927–946.

[21] Junqueira L C, Carneiro J. 12th ed. Rio de Janeiro: Guanabara Koogan; *Histologia básica,* 2013,p. 456-86.

[22] Fessler M B, Rose K, Zhang Y, Jaramillo R , Zeldin D C . Relationship between serum cholesterol & indices of erythrocytes & platelets in the US population. *Journal of Lipid Research,* 2013,**54**(11): 3177-88.

[23] Soetan K O, Akinrinde A S ,Ajibade T O. Preliminary studies on the haematological parameters of cockerels fed raw & processed guinea corn (Sorghum bicolor). *Proceedings of 38th Annual Conference of Nigerian Society for Animal Production.* 2013,Pp. 49-52

[24] Iwuji T C, Herbert U. Haematological & serum biochemical characteristics of rabbit bucks fed diets containing garcimiola kola seed meal. *Proceedings of 37th Annual Conference of Nigerian Society for Animal Production.* 2012, Pp.87-89..

[25] Wang N, Tall AR. Cholesterol in platelet biogenesis & activation. *Blood,* 2016,**127,** 1949–1953.

[26] Dasofunjo K, Timothy F M, Enyam J A ,Asuquo U. U, Usin SG, Ogri M O, Andem I E , Odu F O, Osim M E, Mgbe, PT ,. Haematinic and immunomodulatory effects of leaf extract of G*ongronema latifolium Global Journal of Pure and Applied Sciences* 2023,**29**(2):22-28

[27] Zhang Z, Yang Y, Hill MA, Wu J. Does C-reactive protein contribute to atherothrombosis via oxidant-mediated release of pro-thrombotic factors & activation of platelets? Front. *Journal of Physiology.* 2012, **3,** 433-439.

[28] Braune S, Küpper JH, Jung F. Effect of prostanoids on human platelet function: an overview. International Journal of Molecular Sciences. 2020 Nov 27;21(23):9020.

[29] Peters, S O, Gunn H H, Imumorin I G, Agaviezor, B O , Ikeobi C O. Haematological studies on frizzled & naked neck genotypes of Nigerian native chickens. *Tropical Animal Health Production,* 2011,**43**(3):631-638.

[30] Awodi S, Ayo J O, Atodo A D, Dzende T, 2005. Some haematological parameters & the erythrocyte osomotic fragility in the laughing dove (*Streptopella senegalensis*) and the village weaner bird (Ploceus cucullatus). *Proceedings of the 10th Annual Conference of Animal Science Association of Nigeria.* Pp.384-387.

[31] Chineke C A, Ologun A G, Ikeobi C ON. Haematological parameters in rabbit breeds & crosses in humid tropics. *Pakistan Journal of Biological Sciences,* 2006,**9**(11):2102-2106