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Preventive and Therapeutic Effects of *Garcinia kola* Biflavonoid Fractions on some Haematological Parameters of P407 Induced Hyperlipidemic Albino Rats

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Abstract

The preventive and therapeutic effects of *Garcinia kola* biflavonoid fractions on some haematological parameters in Poloxamer 407 (P407) induced hyperlipidemic rats was studied for a period of 21 days. Sixty mixed sex Wistar rats weighing 150–200 g were divided into two major groups; Preventive group comprising the following sub–groups: normal control, P407 induced hyperlipidemic control and groups treated orally with atorvastatin as standard drug, root bark, stem bark and seed biflavonoid fractions of *Garcinia kola* respectively for 19 days and made hyperlipidemic with a single intraperitoneal injection on day 19. Therapeutic group comprising similar subgroups were induced by an intraperitoneal injection of P407 every 48 hours and treated with atorvastatin, root bark, stem bark and seed biflavonoid fractions for 21 days. In both groups, atorvastatin, P407 and the *Garcinia kola* biflavonoid fractions were administered at 10 mg/kg, 500 mg/kg and 200 mg/kg body weight respectively. Blood samples were collected from the rats in all the groups at the end of the experiment for determination of some haematological parameters levels; Packed Cell Volume (PCV), Platelet, neutrophils, lymphocytes, Haemoglobin (Hb), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Volume (MCV), Red Blood Cell (RBC) Count and White Blood Cell (WBC) Count were determined using the Sysmex Haematological Autoanalyzer. In both groups, atorvastatin and all biflavonoid fractions significantly ($p < 0.05$) reduced the levels of platelet count and had no significant ($p > 0.05$) change on the level of neutrophils compared to hyperlipidemic control. The seed biflavonoid fraction significantly ($p < 0.05$) increased RBC when compared to all the groups except stem bark biflavonoid fraction treated in the therapeutic group. However, there was no significant ($p > 0.05$) change in the levels of the other determined haematological parameters in both groups. These results suggest that *Garcinia kola* (root bark, stem bark and seed) biflavonoid fractions could be potential drugs for the prevention and treatment of platelet–activity thrombosis as well as erythropoietic, protective and stimulating effect.

Keywords: Biflavonoid, *Garcinia kola*, hyperlipidemia, Poloxamer 407, phytopreventive, phytotherapeutic, biochemical parameters

1. Introduction

Haematological parameters are notable features or distinguishing characteristics of the blood and blood forming organs [1]. They are of clinical importance in determining health status. These parameters include: Packed Cell Volume (PCV), Platelet, neutrophils, lymphocytes, Haemoglobin (Hb), Mean Corpuscular

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Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Volume (MCV), Red Blood Cell (RBC) Count and White Blood Cell (WBC) Count. These haematological parameters are associated with diagnosing and evaluating the degree and progression of certain diseases such as hyperlipidemia [2].

Hyperlipidemia is the abnormal elevations of lipids in the blood, largely cholesterol and triglycerides. It is also known as Hyperlipoproteinemia due to abnormal elevations of lipoproteins that transport lipids in the blood [3]. Such high levels of lipids affect the levels of some haematological parameters such as platelet and neutrophil counts, thereby initiating or triggering some symptoms which further enhances/complicates the danger of the disease as it progresses [4].

The influx of Non Esterified Fatty Acids (NEFAs) from the adipocytes increases Tissue Factor (TF) and PAI–1 levels and enhances platelet aggregation; promoting development of thrombosis [5] which contributes to atherosclerosis [6]. Hypercholesterolemia increases circulating inflammatory monocytes counts and renders these cells more prone for emigration into atherosclerotic lesions [7]. Clinical studies correlates systemic neutrophil counts with severity of atherosclerosis in humans supporting an association of neutrophils with disease progression [8].

The complexity of hyperlipidemia, the most common form of dyslipidemia (which also includes any decreased lipid levels) makes it one of the most important risk factors in the development and progression of atherosclerosis leading to Cardiovascular Diseases (CVDs) [9].

Garcinia kola, a dicotyledonous plant belonging to the *Clusiaceae* or *Guttiferae* family is commonly found in the subtropical and tropical forests of some countries of West and Central Africa such as Benin, Cameroon, Democratic Republic of Congo, Ivory Coast, Ghana, Liberia, Nigeria, Senegal and Sierra Leone. The seed is popular and commonly consumed all over Nigeria where it is referred to as 'Bitter Kola' or 'Male Kola' due to its bitter taste and aphrodisiac properties respectively. The plant has been reported to possess a wide range of biological and pharmacological activities; antidiabetic activity [10], antihepatotoxic activity [11], antimicrobial activity [12,13], and antioxidant activity [14]. Therefore, this study was conducted to determine the effect of prophylactic and therapeutic administration of the biflavonoid fractions of the different parts (root bark, stem bark and seed) of *Garcinia kola* on some haematological parameters of P407 induced hyperlipidemic Wistar rats.

2. Materials and Methods

2.1 Experimental Animals

A total of 60 apparently healthy Wistar rats of both sexes weighing between 150–200 g were obtained from National Institute for Trypanosomiasis Research, Kaduna, Nigeria, and kept according to sexes in well aerated laboratory cages in the Animal house, Department of Pharmacology, Ahmadu Bello University, Zaria, Nigeria. The animals were allowed to acclimatize to the laboratory environment for a period of two weeks before the commencement of the experiment (which lasted for 21 days). They were fed with water and grower mash *ad libitum*.

2.2 Collection of Plant Material and Identification

Root bark, stem bark, and seed of *Garcinia kola* were collected from Abak, Akwa Ibom state, Nigeria, in the month of August, 2012. The plant was identified and authenticated at the herbarium unit of Biological Sciences Department, Ahmadu Bello University, Zaria, where voucher specimen number 1783 was given and deposited for future reference.

2.3 Plant Preparation and Extraction

The root bark were washed and sliced, the seeds peeled and sliced and both separately pulverized with an electric blender and air–dried in the laboratory at room temperature alongside the stem bark which was coarsely ground after drying.

The coarsely ground root bark, stem bark and seed were respectively extracted briefly with light petroleum ether (bp 40–60°C) in a Soxhlet Extractor to defat. The defatted, dried residue were repacked and then extracted with acetone. The extracts were concentrated and diluted to twice their volume with distilled water and partitioned with ethylacetate (6×250 ml). The concentrated ethylacetate fraction was considered the biflavonoid fraction. This was further dissolved in 2 to 3 drops of Tween-80 and diluted to the desired concentrations with distilled water to

give a water–soluble fraction. These drug solutions were prepared fresh on the day of experiments [15].

2.4 Acute Toxicity Study (LD_{50})

The mean Lethal Dose (LD_{50}) of *Garcinia kola* (root bark, stem bark and seed) biflavonoid fractions were determined by a method described by Lorke [16].

2.5 Preparation of Standard Drug

Atorvastatin (Pfizer Ireland pharmaceuticals, Ireland) was purchased in a tablet form at strength 20 mg. Tablets were dissolved in distilled water and administered orally.

2.6 Induction of Hyperlipidemia

Poloxamer 407 (Lutrol F127; BASF, Ludwigshafen, Germany) was used as the inducing agent. Prior to the administration, Poloxamer 407 was dissolved in distilled water and refrigerated overnight to facilitate its dissolution. Needles and syringes to be used for administration were also cooled to prevent gelation within the syringe during injection [17].

2.7 Experimental Design

The rats were randomly divided into 2 major groups (Preventive and Therapeutic); with a total of 10 sub–groups comprising of 6 rats each. Groups III to VI were given atorvastatin, root bark, stem bark and seed biflavonoid fractions respectively for 19 days and on the 19th day, injected with Poloxamer 407 (500 mg/ kg b. wt) and sacrificed 48 hrs after (Preventive group). Groups VII to X were administered Poloxamer 407 (500 mg/kg b. wt) at 48 h interval for 21 days; treatment with the respective biflavonoid fraction commenced 2 hours after induction (Therapeutic group).

2.7.1 Preventive Group

- **Group I:** were fed normal chow and distilled water only for 21 days (NC).
- **Group II:** were induced without treatment (HC).
- **Group III:** were treated with Atorvastatin (ATV) at 10 mg/kg body weight/day for 19 days and then induced for 2 days.
- **Group IV:** were treated with Root Bark Biflavonoid Fraction (RBBF) at 200 mg/kg body weight/day for 19 days and then induced for 2 days.
- **Group V:** were treated with Stem Bark Biflavonoid Fraction (SBBF) at 200 mg/kg body weight/day for 19 days and then induced for 2 days.
- **Group VI:** were treated with Seed Biflavonoid Fraction (SBF) at 200 mg/kg body weight/day for 19 days and then induced for 2 days.

2.7.2 Therapeutic Group

- Group I: were fed normal chow and distilled water only for 21 days (NC).
- Group II: were induced without treatment (HC).
- Group VII: were induced and treated with ATV at 10 mg/kg body weight/day for 21 days.
- Group VIII: were induced and treated with RBBF at 200 mg/kg body weight/day for 21 days.
- Group IX: were induced and treated with SBBF at 200 mg/kg body weight/day for 21 days.
- Group X: were induced and treated with SBF at 200 mg/kg body weight/day for 21 days.

The dose regimens were administered per os once daily for the period of the study. The rats were monitored for clinical signs and death.

2.8 Collection and Preparation of Blood Samples for Haematological Analysis

At the end of the 21–day experimental period, the chloroform–inhalation anesthesia was performed on all experimental animals. The anesthetized animals were bled by cardiac puncture. The blood samples were collected into vacutainers containing Ethylene Diamine Tetraacetic Acid (EDTA) for haematological analysis. Packed Cell Volume (PCV), Platelet, neutrophils, lymphocytes, Haemoglobin (Hb), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Volume (MCV), Red Blood Cell (RBC) Count and White Blood Cell (WBC) Count were determined using the Haematological Autoanalyzer (Beckman Coulter, Inc. Fullerton, CA, USA).

2.9 Data Analysis

Data were expressed as mean±Standard Deviation (SD) and were analyzed by the Analysis of Variance (ANOVA) with the aid of the Statistical Package for Social Science

version 17.0. The difference between the various biflavonoid fractions and animal groups were compared using the Duncan Multiple Range Test. p value less than 0.05 was considered significant ($p < 0.05$).

3. Results

3.1 Effects of Garcinia kola Biflavonoid Fractions on Some Haematological Parameters of P407 Induced Hyperlipidemic Rats

Tables 1 and 2 show the preventive and therapeutic effects of oral administration of *Garcinia kola* biflavonoid fractions on some haematological parameters of P407 induced hyperlipidemic rats respectively. The results showed no significant ($p > 0.05$) difference in the levels of Haemoglobin (Hb), lymphocytes, Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Volume (MCV), Packed Cell Volume (PCV) and White Blood Cell (WBC) count in all the groups. Hyperlipidemia significantly ($p < 0.05$) increased platelet count compared to control and treated group (Tables 1 and 2). There is insignificant ($p > 0.05$) decrease in the level of neutrophils of all treated groups compared to hyperlipidemic group (Tables 1 and 2). However, therapeutic administration of the seed biflavonoid fraction significantly ($p < 0.05$) increased RBC when compared to all the groups except stem bark biflavonoid fraction treated group (Table 2) while prophylactic administration of *G. kola* biflavonoid fractions had no significant effect on RBC of all the groups (Table 1).

4. Discussion

Assessment of haematological parameters can be used not only to determine the extent of deleterious effect of a disease on the blood of an animal, but also to explain blood relating functions of a plant part or its extract [18]. Haematological evaluation in this study showed a significant ($p < 0.05$) increase in the level of platelet count in hyperlipidemic control compared to normal control (Tables 1 and 2). The high level of platelet count could be attributed to increase platelet aggregation, resulting in influx of platelet from the bone marrow due to increased thromboxane A_2 biosynthesis [19] which is in agreement with other studies [5, 20]. However, the *Garcinia kola* (root bark, stem bark and seed bark) biflavonoid fractions significantly ($p < 0.05$) reduced the level of platelet count compared to the hyperlipidemic control. The *Garcinia kola* biflavonoid fractions could have done this by lowering intracellular Ca^{2+} levels; altering the metabolism of CAMP thereby inhibiting thromboxane A_2 formation which was stimulated by high levels of cholesterol as suggested by Roy et al. [21] and Sandhar et al. [22]. Thus preventing or reducing thrombosis. This is an additional benefit in the use of *Garcinia kola* in managing cardiovascular diseases and in agreement with studies that have shown flavonoids prevent platelet activity-related thrombosis [23].

The seed biflavonoid fraction significantly ($p < 0.05$) increased red blood cell count (RBC) compared to all the groups except the stem bark fraction (Table 2). This could be that the stem bark and seed biflavonoid fractions stimulate the cytokine erythropoietin which increases RBC lines as reported by Ahumibe and Braide [24] which is based on the antioxidant property of *Garcinia kola* [25] thereby elevating the total antioxidant capacity of the blood. This implies that the stem bark and seed biflavonoid fractions have erythropoietic, protective and stimulating effect validating the use of the seed extracts in antisickling studies [26].

This research also showed significant ($p<0.05$) increase in neutrophils level of the hyperlipidemic control group (Tables 1 and 2) compared to normal control. Hypercholesterolemia enhances serum CXCL1 levels, which promotes neutrophil mobilization via CXCR2. Hence, hyperlipidemia disturbs the tightly regulated cytokine system controlling neutrophil homeostasis at various levels, ultimately increasing peripheral neutrophil counts [27]. This also buttresses the successful induction of hyperlipidemia in this study. Although, *Garcinia kola* fractions insignificantly ($p>0.05$) decreased neutrophil levels, an increase in concentration or duration of treatment could be of advantage in preventing or reducing the risk of atherosclerosis which is associated with high level of neutrophils [8].

5. Conclusion

The findings of this study have shown that *Garcinia kola* (root bark, stem bark and seed) biflavonoid fractions

Table 1: Phytopreventive effect of *Garcinia kola* biflavonoid fractions on some haematological parameters of wistar rats

Group (n=6)	Hb (mg/dl)	Lym (%)	Neut (%)	MCH (pg)	MCHC (g/dl)	MCV (fl)	PCV (%)	PLT ($\times10^3$/ul)	RBC ($\times10^6$/ul)	WBC ($\times10^3$/ul)
NC	13.53 ± 1.22^a	81.80 ± 6.31^a	5.13 ± 0.46^a	20.43 ± 1.15^a	33.23 ± 1.02^a	63.13 ± 3.46^a	42.17 ± 2.31^a	601.43 ± 64.90^a	6.46 ± 0.29^a	17.97 ± 2.08^a
P407	13.23 ± 1.04^a	77.47 ± 0.60^a	7.43 ± 0.98^b	20.33 ± 1.23^a	33.03 ± 1.27^a	61.53 ± 2.97^a	40.10 ± 1.97^a	1154.00 ± 25.36^d	6.11 ± 0.27^a	19.60 ± 1.11^a
P407+ ATV	13.13 ± 0.51^a	82.07 ± 9.55^a	6.59 ± 0.70^{ab}	19. 37 ± 1.31^a	32.67 ± 0.06^a	59.33 ± 4.06^a	40.20 ± 1.54^a	772.00 ± 80.52^{bc}	6.81 ± 0.70^a	18.17 ± 1.45^a
P407+RBBF	13.07 ± 0.19^a	72.50 ± 0.26^a	5.24 ± 0.80^{ab}	19.20 ± 0.78^a	32.63 ± 1.12^a	58.80 ± 0.96^a	40.10 ± 1.81^a	792.00 ± 19.05^c	6.82 ± 0.83^a	17.73 ± 3.64^a
P407+SBBF	12.27 ± 0.29^a	82.83 ± 6.31^a	5.45 ± 1.10^{ab}	18.53 ± 0.65^a	32.27 ± 0.72^a	57.40 ± 1.12^a	38.67 ± 1.16^a	651.33 ± 99.00^{ab}	6.62 ± 0.12^a	18.17 ± 2.48^a
P407+SBF	13.23 ± 0.55^a	81.93 ± 2.80^a	5.67 ± 1.50^{ab}	19.57 ± 1.86^a	32.20 ± 0.35^a	60.75 ± 5.06^a	41.10 ± 1.34^a	744.33 ± 93.09^{ab}	6.80 ± 0.56^a	17.60 ± 1.65^a

Values are means ± Standard deviations

Values with different superscripts down the column differ significantly ($p<0.05$)

NC: Normal Control Rats, P407: Poloxamer 407 Induced Rats, ATV+P407: 10mg of Atorvastatin+P407 Induced Hyperlipidemic Rats, RBBF+P407: 200mg of Root bark Biflavonoid Fraction+P407 Induced Hyperlipidemic Rats, SBBF+P407: 200mg of Stem bark Biflavonoid Fraction+P407 Induced Hyperlipidemic Rats, SBF+P407: 200mg of Seed Biflavonoid Fraction+P407 Induced Hyperlipidemic Rats

Hb: Haemoglobin, Lym: Lymphocytes, Neut: Neutrophils, MCH: Mean Corpuscular Haemoglobin, MCHC: Mean Corpuscular Haemoglobin Concentration, MCV: Mean corpuscular volume, PCV: Packed Cell Volume, PLT: Platelet, RBC: Red Blood Cell Count, WBC: White Blood Cell Count

Table 2: Phytotherapeutic effect of *Garcinia kola* biflavonoid fractions on some haematological parameters of wistar rats

Group (n=6)	Hb (mg/dl)	Lym (%)	Neut (%)	MCH (pg)	MCHC (g/dl)	MCV (fl)	PCV (%)	PLT ($\times10^3$/ul)	RBC ($\times10^6$/ul)	WBC ($\times10^3$/ul)
NC	13.53 ± 1.22^a	81.80 ± 6.31^a	5.13 ± 0.46^a	20.43 ± 1.15^a	33.23 ± 1.02^a	63.13 ± 3.46^a	42.17 ± 2.31^a	601.43 ± 64.90^a	6.46 ± 0.29^{ab}	17.97 ± 2.08^a
P407	13.23 ± 1.04^a	77.47 ± 0.60^a	7.43 ± 0.98^b	20.33 ± 1.23^a	33.03 ± 1.27^a	61.53 ± 2.97^a	40.10 ± 1.97^a	1154.00 ± 25.36^c	6.11 ± 0.27^a	19.60 ± 1.11^a
P407+ ATV	11.40 ± 2.69^a	75.27 ± 12.08^a	7.10 ± 1.41^{ab}	20.23 ± 0.64^a	32.33 ± 1.00^a	62.35 ± 2.20^a	37.20 ± 6.32^a	815.33 ± 56.58^b	6.16 ± 0.14^a	16.87 ± 1.37^a
P407+RBBF	13.17 ± 0.15^a	81.37 ± 8.23^a	6.40 ± 1.21^{ab}	20.33 ± 0.15^a	32.37 ± 1.14^a	61.90 ± 1.87^a	40.70 ± 1.98^a	723.00 ± 45.03^b	6.44 ± 0.05^{ab}	16.40 ± 4.37^a
P407+SBBF	11.40 ± 3.05^a	76.13 ± 7.92^a	7.10 ± 0.28^{ab}	19.80 ± 2.00^a	31.03 ± 0.97^a	63.77 ± 4.49^a	39.13 ± 3.78^a	612.00 ± 68.46^a	6.90 ± 0.22^{bc}	16.30 ± 1.52^a
P407+SBF	13.33 ± 0.46^a	82.83 ± 6.90^a	6.40 ± 1.21^{ab}	18.73 ± 0.95^a	31.03 ± 1.18^a	60.47 ± 0.87^a	41.07 ± 3.09^a	786.67 ± 23.96^b	7.12 ± 0.59^c	17.83 ± 2.04^a

Values are means ± Standard deviations

Values with different superscripts down the column differ significantly ($p<0.05$)

NC: Normal Control Rats, P407: Poloxamer 407 Induced Rats, P407+ATV: P407 Induced Hyperlipidemic Rats+10mg of Atorvastatin, P407+RBBF: P407 Induced Hyperlipidemic Rats+200mg of Root bark Biflavonoid Fraction, P407+ SBBF: P407 Induced Hyperlipidemic Rats+200mg of Stem bark Biflavonoid Fraction, P407+SBF: P407 Induced Hyperlipidemic Rats+200mg of Seed Biflavonoid Fraction

Hb: Haemoglobin, Lym: Lymphocytes, Neut: Neutrophils, MCH: Mean Corpuscular Haemoglobin, MCHC: Mean Corpuscular Haemoglobin Concentration, MCV: Mean corpuscular volume, PCV: Packed Cell Volume, PLT: Platelet, RBC: Red Blood Cell Count, WBC: White Blood Cell Count

may be efficacious as potential drugs for the prevention and treatment of platelet-activity thrombosis and erythropoietic, protective and stimulating effect by the stem bark and seed biflavonoid fractions.

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