Nigerian Journal of Biochemistry and Molecular Biology
Volume 26, No. 1, 2011. pp. 23-28 (Printed in Nigeria)
0189-4757/96 $3.0 + 0.00

NJBMB/019/11

Phytochemical Constituents and Haematological Profile of Male Rats Following Oral Administration of Aqueous Extract of *Garcinia kola* (Heckel) Seeds

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ABSTRACT: The phytochemical screening and the effect of administration of aqueous extract of *G. kola* seeds at the doses of 25, 50 and 100 mg/kg body weight on daily basis for 7days on haematological profile of male Wistar rats was investigated. Male albino rats (215.00± 18.58 g) were grouped into four (A-D) such that animals in group A received 0.5 ml of distilled water while those in groups B, C, and D received same volume of the extract corresponding to 25, 50 and 100 mg/kg body weight respectively. The extract contained saponin (2.78%), steroids (1.14%), flavonoids (1.28%), cardiac glycosides (0.26%), cardenolides and dienolides (0.24%) while tannins, anthraquinones, phenolics, phlobatanins and triterpenes were absent. All the doses of the extract significantly decreased ($P<0.05$) the levels of Haemoglobin (Hb), Packed Cell Volume (PCV), and Red Blood Cells (RBC). In contrast, the levels of White Blood Cell (WBC) and platelets increased significantly ($P<0.05$). While the levels of Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), and Mean Corpuscular Haemoglobin Concentration (MCHC) were not significantly altered ($P>0.05$) by all the doses of the extract, there was dose specific effects on the neutrophils and lymphocytes of the animals. Findings from this study have revealed that the administration of the aqueous extract of *G. kola* seeds at the doses of 25, 50 and 100 mg/kg body weight once daily for 7 days is haematotoxic and therefore not safe at these doses for consumption.

KEYWORDS: *Garcinia kola*, Guttifera, Haematotoxic, Phytochemicals

INTRODUCTION

Medicinal plants typically contain several different pharmacologically active compounds that may act individually, additively or in synergy to improve health (Azaizeh *et al*., 2003; Gurib-Fakim, 2006). Plants produce these bioactive compounds which act as defence mechanisms against predators and at the same time, may be toxic in nature (Da Roch *et al*., 2001; Bent and Ko, 2004). With the upsurge of interests in medicinal plants, there is the need therefore for thorough scientific investigations of these plants for both efficacy and potential toxicity (Ashafa *et al*., 2010).

Garcinia kola Heckel (Family - *Guttifera*) commonly known as bitter kola, male kola and false kola (English) is also known by various tribes in Nigeria as *Orogbo* (Yoruba), *Cida goro* (Hausa), *Aku ilu* or *Ugugolu* (Igbo), *Efiari* (Efik), and *Igoligo* (Idoma).

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It occurs naturally in Sierra Leone, Angola and Nigeria. It is an evergreen, unbuttressed, heavily crowned dicotyledonous plant found in moist forest as a medium sized tree which is about 13-15 m high.

The plant has been acclaimed to be used in the management of liver disorders and diarrhoea (Iwu *et al.*, 1990; Blaide, 1991), diabetes, bronchitis and throat infections (Tita *et al*., 2001; Orie and Ekon, 1993) and as an aphrodisiac.

There have been scientific reports that have lent support to the consumption of *G. kola* seeds as a stimulant (Atawodi *et al*., 1995) and hepatoprotective activity against paracetamol-induced hepatotoxicity in rats and atherogenic effect in hypercholesterolaemia rats (Akintonwa and Essien, 1990; Adaramoye *et al*., 2005; Ajiboye and Satake, 1992). The seeds are known to contain a high amount of bioflavonoids (Iwu, 1986) and the antioxidant activity of the flavonoids from *G. kola* seeds have also been documented (Emerole *et al*., 2005).

Despite the acclaimed myriad of uses of *G. kola* seeds in the folklore medicine of Nigeria, the effects of the seed extract on the haematological parameters (Hb, PCV, RBC, MCV, MCH, MCHC, WBC, platelets, neutrophils and lymphocytes) of male rats has not been reported in the open scientific literature.

Therefore, this study was aimed at investigating the phytochemical constituents and the effects of the aqueous extract of the *G. kola* seeds on haematological parameters of male Wistar rats.

MATERIALS AND METHODS

Plant materials and authentication

Garcinia kola seeds purchased at a market (Agor Market), Ilorin, Nigeria, was authenticated at the Forestry Research Institute of Nigeria, Jericho, Ibadan, Nigeria. A voucher specimen (FHI 10847) was deposited at the herbarium of the Institute.

Experimental animals

Twenty four albino rats (*Rattus norvergicus*) weighing 215.00 ± 18.58 g obtained from the Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria, were maintained under standard housing conditions (temperature: 22±3°C; photoperiod: 12 h natural light and 12 h dark; humidity: 50-55%). The animals were allowed free access to rat pellets (Bendel Feeds and Flour mill Ltd, Ewu, Nigeria) and tap water throughout the period of the study.

Preparation of aqueous extract of G. kola seeds

The seeds were peeled, cut into pieces and oven-dried at 40°C for 24h to a constant weight using Uniscope Laboratory Oven, (SM9053 Surgifriend Medicals, England). The dried pieces were then pulverized using an electric blender (Crownstar Blender CS- 242B, Trident (H.K) Ltd, China.

A known amount (300 g) of the powder was extracted in 1000 ml of distilled water for 48 h at room temperature. This was then filtered using Whatman No 1 filter paper. The filtrate was concentrated on a steam bath to give 16.42 g of the residue. This residue was reconstituted in distilled water to give the required doses of 25, 50 and 100 mg/kg body weights used in this study the choice of which was as a result of the findings from ethnobotanical survey.

Phytochemical screening

Standard laboratory methods as described for alkaloids, steroids, anthraquinones, cardenolides and dienolides (Trease and Evans, 1986), saponins, phenolics, flavonoids, cardiac glycosides, triterpenes and tannins (Odebiyi and Sofowora, 1978), were used in detecting the presence of phytochemicals in the plant.

Animal grouping

Twenty four healthy Wistar rats were randomly grouped into four (A-D) of six rats each. Rats in group A (control) received 0.5 ml of distilled water by means of oropharygeal cannula. Those in groups B-D were administered 0.5 ml of 25, 50 and 100 mg/kg body weights of the extract respectively. The treatments were done once daily for seven days.

Collection of blood and analysis of haematological parameters

On day 7, the rats were sacrificed, their jugular veins were cut and blood samples collected into sample bottles containing EDTA was analyzed for haematological indices such as PCV, RBC and WBC (Schalm *et al*., 1975), Hb and platelets (Kelly, 1979). The MCV, MCH and MCHC were computed using appropriate formula as described by Jain (1986).

Statistical analysis

Results were expressed as mean ± SD. Statistical analysis was performed by one-way analysis of variance (ANOVA). Student's t-test at 95% level of significance was used to assess significant difference between the control and treated groups.

RESULTS

Phytochemical screening revealed the presence of cardiac glycosides, flavonoids, steroids, saponins and cardenolides and dienolides (Table 1). Quantitatively, saponin showed the highest concentration followed by flavonoids and steroids, while cardiac glycosides, cardenolides and dienolides were weakly present. However, tannins, anthraquinones, phenolics, alkaloids, phlobatanins and triterpenes were absent (Table 1).

All the doses of the extract significantly decreased the levels of Hb, PCV, and RBC of the animals. In contrast, the levels of WBC and platelets increased significantly ($P<0.05$). Furthermore, the levels of MCV, MCH, and MCHC were not significantly altered ($P>0.05$) by all the doses of the extract. The extract also dose specifically affected the neutrophils and lymphocytes of the animals. For instance, while the doses of 25 and 50 mg/kg body weight did not significantly alter the neutrophils and lymphocytes, the 100 mg/kg body weight significantly increased the neutrophil count to about 2.4 fold the control. In addition, the same dose decreased the lymphocyte count of the animals (Table 2).

Table 1: Phytochemical constituents of aqueous extract of *Garcinia kola* seeds

Phytochemicals	Concentrations (%)
Tannins	Absent
Anthraquinones	Absent
Phenolics	Absent
Cardiac glycosides	0.26±0.02
Flavonoids	1.28±0.01
Steroids	1.14±0.05
Saponins	2.78±0.03
Alkaloids	Absent
Cardenolides and dienolides	0.24±0.03
Phlobatannins	Absent
Triterpenes	Absent

Data in triplicates ± S.D

DISCUSSION

Medicinal plants or drugs can alter a wide range of haematological parameters (Ajagbonna *et al*., 1999) and this may be due largely to difference in the biological functions of the components of such plants. Hematological parameters are good indicators of the physiological status of animal and alterations in the indices could be used to assess the response of animals to various physiological situations (Esonu *et al*., 2006). Changes in blood parameters can also be used to explain blood-relating functions of the plant extract or its products (Yakubu *et al*., 2007).

Therefore, the reduction in RBC by the extract in the present study may be an indication the erythropoiesis was adversely affected. The extract might have interfered with the balance between the rate of production and destruction of the blood corpuscles (Yakubu and Afolayan, 2009). The accompanying decrease in HB and PCV is quite understandable since they are directly related to the RBC. Therefore, the release of erythropoietin in the kidney which is the humoral regulator of RBC production was adversely affected (Sanchez-Elsner *et al*., 2004).

Table 2: Effect of aqueous extract of *G. kola* seeds on haematological parameters in male rats

	Haematological Parameters									
Doses (mg/kg body weight)	**Hb (g/dl)**	**PCV (%)**	**RBC $\times 10^{12/1}$**	**MCV (fl)**	**MCH (pg)**	**MCHC g/dl**	**WBC $\times 10^{g/l}$**	**Platelets $\times 10^{g/l}$**	**Neutrophils (%)**	**Lymphocytes (%)**
Control	16.03±0.94[a]	53.0±1.10[a]	4.06±0.04[a]	130.5±1.64[a]	38.0±1.10[a]	34.6±0.89[a]	7.2±1.10[a]	840.0±54.77[a]	12.0±0.00[a]	88.0±0.00[a]
25	13.05±1.15[b]	44.0±1.10[b]	3.18±0.00b[b]	126.0±8.96[a]	37.5±0.55[a]	34.0±1.79[a]	9.2±1.53[b]	905.0±16.43[b]	11.0±1.10[a]	89.0±1.10[a]
50	12.75±1.59[b]	43.0±5.48[b]	3.86±0.066[b]	123.0±10.95[a]	36.0±1.29[a]	35.3±1.37[a]	9.0±0.16[c]	935.0±16.43[c]	13.0±1.10[a]	87.0±1.10[a]
100	15.2±1.10[c]	51.5±3.85[c]	3.26±0.26[c]	125.5±3.83[a]	37.0±0.10[a]	34.0±0.00[a]	8.7±0.17[d]	985.0±5.48[d]	29.0±7.67[b]	71.0±7.67[b]

n=6 ± SD, Superscripts a, b, c, and d are significantly different from the control group

Saponins have been reported to have haemolytic properties (Okwu, 2005). Therefore, the presence of saponins in the extract may be responsible for the reduction in RBC and consequently the drop in PCV and Hb. The reduction in the blood parameters will have consequential effects on their normal function in the animals. MCV, MCH and MCHC are related to individual red blood cells. Therefore, the lack of an on these haematological parameters suggests that the individual red blood cells in terms of their average size (microcytes) and weight of Hb per RBC were not affected (McLellan *et al*., 2003), but the total population of RBC as evidenced by the decrease in RBC, Hb and PCV.

WBC, lymphocytes and neutrophils are immunologic machines of the body against infections. Alterations in these haematological parameters may lead to immunologic incapacitation. The increase in WBC may suggest that the rate of entrance of the corpuscle into the blood from the bone marrow was enhanced over its rate of removal from circulation. It is also possible that the haematopoietic regulatory elements of the stromal cells and macrophages in the bone marrow were enhanced. This is further corroborated by the stimulatory effect of the extract on thrombopoietin which is directly related to platelet production (Li *et al*., 1999). Since WBC increase the immunological action of the body by fighting antigens either by phagocytosis or cytotoxicity, the significant increase in WBC following the administration of the extract at all the doses suggests a boost in the immune system of the animals.

The reduction in lymphocyte counts at 100 mg/kg body weight and lack of an effect at the lower doses of 25 and 50 mg/kg body weight of the extract may indicate parameter- and dose-specific effect of the extract. This may also explain the effect on the lymphocyte at only 100 mg/kg body weight of the extract among the other doses investigated in the present study.

In conclusion, this study has revealed that the aqueous extract of the seeds of *G. kola* at the doses of 25, 50 and 100 mg/kg body weight selectively altered the levels of the haematological parameters of the animals. Therefore, the extract has exhibited selective haematotoxicity on the animals.

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