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EFFECT OF GAVAGE TREATMENT WITH PULVERISED *GARCINIA KOLA* SEEDS ON ERYTHROCYTE MEMBRANE INTEGRITY AND SELECTED HAEMATOLOGICAL INDICES IN MALE ALBINO WISTAR RATS

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Summary: This study examines the effect of the whole seed of *Garcinia kola (GKS*) on various blood parameters, in adult male albino rats. Five groups, of 6 animals per group, were treated by gavage with suspensions of graded concentrations of GKS daily for 5 weeks. The animals were then sacrificed and blood was obtained for estimation of the data herein presented. Packed red cell volume (PCV), hemoglobin concentration (Hb), and red blood cell count (RBC) showed significantly ($P < 0.05$, Student's t-test) increased response to treatment with GKS; while the platelet and white blood cell (WBC) counts showed no corresponding increase with increasing GKS dosage. The mean red blood cell volume (MCV) and mean cell hemoglobin (MCH) levels decreased with increasing GKS dosage. Prothrombin time (PT) and activated partial thromboplastin time (APPT) were both prolonged with increased GKS dosage; while the serum lipids (cholesterol and triglycerides) decreased significantly ($P< 0.05$, Student's t-test) with increased GKS dosage.

Key Words: *Garcinia kola; erythrocyte osmotic fragility; haematological parameters; serum cholesterol triglycerides*

Introduction

One of many plant sources of therapeutic ingredient among the people in West and Central Africa and some parts of Asia, is the seed plant *Garcinia kola* Heckel (Guttiferae). It is a predominant source of natural *materia medica* employed for the prophylactic management of disease (Konoshima *et al*., 1970). In the literature of ethnopharmacology, *Garcinia kola* is credited with numerous therapeutic applications: poison antidote (Dalziel, 1937); treatment of diarrhea, hepatitis, asthma, dysmenorrhea or menstrual cramps (Dalziel 1956); gonorrhea (Otung, 1990).

The *Garcinia kola* seed ("bitter kola") is ingested in relatively large amount at various social gatherings; and a number of studies have been done while pondering over its possible toxicity in humans vis-à-vis the pharmacotherapeutic effects of this plant product. It has been shown that chronic ingestion of the "bitter kola" causes histological changes in the liver, kidney and gastrointestinal tract of rats (Braide 1990; Braide and Grill, 1990). Most of the studies on the pharmacological activity of phytochemical components of *Garcinia kola* seed, have been on the biflavonoids. These phytochemicals have been shown to have a very broad spectrum of pharmacological activity: protective action against chemical induced hemolysis in G6PD deficient human red blood cells (Sharma *et al*, 2003); anti-inflammatory antipyretic activity (Braide, 1993; Olaleye, 2000) inhibition of hepatic drug metabolism (Braide, 1991); antihepatotoxicity (Akintonwa and Essien, 1990; Iwu, 1985, Braide, 1991a; Adegoke *et al*,1998; Adaramoye and Akinloye, 2000; Farombi, 2000; Farombi *et al*, 2000); hypoglycaemic anti-diabetic activity (Iwu *et al*, 1990); bronchodilatation in man (Orie and Ekon 1993); antispasmodic effect on smooth muscle (Braide, 1989); anti-thrombotic effect (Olajide, 1999).

The objective of this study is to investigate the effect of *Garcinia kola* seed on the erythrocyte membrane integrity, hematological cell indices and selected coagulation protein properties. The following questions shall be addressed: Can the "bitter kola" affect erythrocyte membrane integrity? This question can be answered, after monitoring erythrocyte responses to osmotic fragility testing. Can the "bitter kola" have any effect on the extrinsic and intrinsic coagulation pathways, as represented by the measure of prothrombin time (PT) and activated partial thromboplstin time (APTT)? The answer to this question may help to establish any linkages between *Garcinia kola* and the coagulation proteins. What is the effect of *Garcinia kola* on the numbers and concentrations of blood formed elements on the one hand, and the hemoglobin concentration of the red blood cells on the other hand?

Materials and Methods

Seeds of Garcinia kola

Garcinia kola seeds were purchased in season from markets in Karu and New Wuse, in the Federal Capital Territory of Abuja. Confirmation of the plant material was done at the National Institute of Pharmaceutical Research and Development (NIPRD) Idu, Abuja, Nigeria.

Preparation of Seed Samples

The fresh seeds of *Garcinia kola* were peeled to remove the testa, washed, pelleted and air-dried for 8h, then subsequently dried in an electric oven for 12h at 30^{o}C. The dry seed pellets were ground to a fine powder and stored in a dry glass bottle, from which aliquots were reconstituted with distilled water to obtain suspensions of appropriate concentrations for oral administration.

Animals

Male Wister albino rats (weighing 200-250g) purchased from the National Veterinary Research Institute (NVRI) Vom, near Jos, Plateau State, Nigeria, were used for this study. The rats were randomly assigned to five groups of six animals each, housed in metal cages, and allowed an acclimatization period lasting 10 days, prior to use for any experiments. The animals had *ad libitum* access to water and standard laboratory animal feed (NIPRD Animal Feed).

Administration of Garcinia kola Seed Powder

Animals in all groups, excepting the controls, received by gavage graded concentrations of *G.kola* seed powder (suspended in distilled water) daily for 35 days. Four doses were administered to four different groups of experimental animals (300mg/kg, 600mg/kg, 900mg/kg and 1200mg/kg).

Collection and Handling of Blood Samples

All animals were sacrificed at the end of 35 days of treatment with *G.kola* seed powder suspension, and whole blood was obtained via cardiac puncture. Aliquots the blood were distributed into various suitable containers for subsequent analysis as follows: 0.2ml of blood into vial containing 0.1mg Na_2 EDTA anticoagulant [for Hb assay; for counts of various blood cells – RBC, WBC, platelets, differential; and for packed cell volume estimation]; 2.5ml of blood into vial containing 0.3 ml of 3.2% trisodium citrate [for preparation of platelet – poor plasma (PPP); 2.0ml of blood into vial containing Heparin anticoagulant [for erythrocyte osmotic fragility test]; and 1.0ml of blood into plain vial [for estimation of serum cholesterol and triglycerides].

Estimation of Packed Cell Volume (PCV)

Blood sample anticoagulated with EDTA was made to enter a plain glass capillary tube, one end of which was later sealed with non absorbent sealer clay. The tube was then spun at 11,000 rpm for 5 min in a microhaematocrit centrifuge. The PCV value was then read, using a microhaematocrit reader.

Estimation of Blood Cell Parameters

Haemoglobin (Hb) concentration, red blood cell (RBC) count, white blood cell (WBC) count, platelet count, mean corpuscular volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) were estimated using a semiautomatic haemotological analyzer (SWELAB IEO Model). The auto counter utilized 20µl of blood in 16ml of a commercially prepared diluent. The machine's ability to count cells was based on the principle of electronic impedance.

Estimation of Prothrombin time (PT)

An aliquot (0.1ml) of platelet – poor plasma (PPP) sample from each animal was dropped individually into test tubes numbered 1 to 30. Thereafter, 0.1ml of a commercially prepared control sample was dropped into tube number 31. All the tubes were warmed at 37^{o}C for 3 min (tube No. 31 for 5 min). Subsequently, 0.2ml of prothrombin thromboplastin reagent was added to each test tube; and the clotting time was recorded for each tube.

Estimation of Activated Partial Thromboplastin time (APPT)

An aliquot (0.1ml) of APTT reagent (Darlez Nig. Ltd, Abuja) was added to a pair of test tubes (one containing a test sample and the other serving as control). The tubes were incubated at 37^{o}C for 5min, before adding 0.1ml $CaCl_2$ solution (Darlez Nig. Ltd, Abuja). The clotting time was noted.

Ertythrocyte Osmotic Fragility Test

This test was done on all rats in each group; and blood samples collected in heparinised vials were analyzed within 3h of collection. A commercially prepared 10% buffered saline working solution was used. The red blood cell sample was washed three times in isotonic saline, after which 0.02ml of each washed cell sample was placed in eight graded concentrations of working saline solution [0.1%, 0.25%, 0.30%, 0.35%, 0.40%, 0.55%, 0.65% and 0.85] and incubated at 37^{o}C for 30min, then centrifuged for 5min. The optical absorbance of each supernatant was then measured, using a colorimeter, at 540nm wavelength. The percentage haemolysis was calculated, for each of the blood samples obtained, and used to develop an osmograph for evaluating the osmotic fragility.

Estimation of Serum Cholesterol

The enzymatic estimation of serum cholesterol was done using a chemistry analyzer (Humalyser 2000). The basic principle is dependent on enzymatic hydrolysis and oxidation of cholesterol to produce red quinoximine, the color intensity of which is proportional to the total serum cholesterol, using the procedure outlined in a commercially prepared kit.

Results

Treatment, by gavage, with pulverized *Garcinia kola* seed (GKS) produced various effects on blood parameters in albino rats. These effects included changes in hematological indices (Hb, PCV, MCV, MCH and MCHC, RBC, WBC, PT APPT); alterations in blood chemistry (serum cholesterol and tryplyceride) and Erythrocyte hemolysis in various concentrations of hypotonic saline solutions [an indication of degree of osmotic fragility]; all of which appeared to be dose-dependent.

Rats treated with various doses of GKS showed significant ($p<0.05$, Student's t-test) dose-dependent increase in haemoglobin (Hb) concentration, packed cell volume (PCV) and mean corpuscular haemoglobin concentration (MCHC). On the other hand, the mean cell volume (MCV) and mean cell haemoglobin (MCH) showed a dose-dependent depreciation (Table 1). Rats that received GKS at doses of 600mg/kg, 900mg/kg and 1200mg/kg, respectively exhibited significant ($p<0.05$, Student's t-test) increments in red blood cell (RBC) count, but the platelet numbers depreciated significantly ($p<0.05$, Student's t-test). (Table 2).

Representative parameters of the blood coagulation mechanism investigated in this study, namely, prothrombin time (PT) and activated partial thromboplastin time (APPT), indicated that both PT and APPT exhibited dose-dependent increases (Table 3). The effect of GKS therapy on serum cholesterol and triglyceride was a dose-dependent decrease in these parameters (Table 4).

Effect of GKS therapy on Erythrocyte Osmotic Fragility

Erythrocytes of the untreated control rats (0mg/kg GKS) exhibited the earliest sign of haemolysis in 0.65% NaCl solution; whereas the erythrocytes from rats that received GKS therapy at doses of 300mg/kg and 600mg/kg exhibited 5% and 3% haemolysis when placed into 0.55% NCl solution. A corresponding onset of haemolysis in rats that received GKS therapy at doses of 900mg/kg and 1,200mg/kg was observed when the representative erythrocytes were placed in 0.40% NaCl solution and 0.35% NaCl solution, respectively. At 0.1% NaCl solution, the erythrocytes from all groups, including untreated controls, exhibited 100% haemolysis.

Table 1: Mean values of HB. PCV. MCV. MCHC for graded doses of GSK administered among the groups.

Index	Dose of GKS powder				
	Control	*300mg/kg*	*600mg/kg*	*900mg/kg*	*1200mg/kg*
HB	12.34± 0.17	12.87± 0.16	13.28 ± 0.12[a]	13.48 ± 0.11[a]	13.45 ± 0.20[a]
PCV	45.34± 0.55	45.83± 0.44	47.12 ± 0.24[a]	47.65 ± 0.25[a]	48.01 ± 0.36[a]
MCV	64.80± 0.97	61.83± 1.40	61.6 ± 0.16[a]	60.63 ± 0.09[a]	57.83 ± 1.30[a]
MCH	17.71 ± 0.22	17.30 ± 0.33	17.25 ± 0.35	16.80 ± 9.41	17.26 ± 0.15
MCHC	27.25 ± 0.48	28.01 ± 0.40	28.29 ± 0.32	28.55 ± 0.25	28.36± 0.43

a =significantly difference from control ($p< 0.5$, Student's t-test)

Table 2: Mean values of RBC, WBC & Platelet count for graded doses of GSK administered among the groups.

Index	Dose of GKS powder				
	Control	*300mg/kg*	*600mg/kg*	*900mg/kg*	*1200mg/kg*
RBC	6.99.0.09	7.46 ± 0.17[a]	7.72 ± 0.15[a]	7.87 ± 0.160[a]	8.32 ± 0.14[a]
WBC	81.67 ± 7.25	81.67 ± 6.17	79.33 ± 5.63	81.5 ± 4.25	78.00 ± 4.04
Platelets	686.67 ± 9.34	636.67± 25.71	618.17 ± 25.24	692 ± 21.51	677.67 ± 23.63

a = significantly different from control ($p <0.05$, Student's t-test).

Table 3: Mean values of PT & APPT for graded doses of GSK Powder Administered among groups.

Index	Dose of GKS powder				
	Control	*300mg/kg*	*600mg/kg*	*900mg/kg*	*1200mg/kg*
PT	6.13 ± 0.32	7.63 ± 0.49[a]	8.58 ± 0.22[a]	8.35 ± 0.30[a]	9.62 ± 0.52[a]
APPT	16.47 ± 2.77	19.65 ± 0.95	21.28 ± 52[a]	21.92 ± 0.75[a]	23.27 ± 0.73[a]

a = significantly different from control ($p<0.05$, Student's t-test).

Table 4: *Mean values of triglycerides and cholesterol for graded doses of GKS administered among groups.*

Index	Dose of GKS powder				
	Control	*300mg/kg*	*600mg/kg*	*900mg/kg*	*1200mg/kg*
Triglyceride	1.23 ± 0.13	0.98 ± 0.11	0.94 ± 0.10 [a]	0.76 ± 0.07 [a]	0.75 ± 0.15[a]
Cholesterol	5.98 ± 0.16	5.39 ± 0.39	5.39 ± 0.28 [a]	4.86 ± 0.35 [a]	4.60 ± 0.33[a]

a = significantly different from control ($p<0.05$, Student's t-test).

Table 5: *Mean values of Osmotic haemolysis of Erythrocytes along grade doses of GKS powder feed.*

% NaCl	%Haemolysis				
	Group A	*Group B*	*Group C*	*Group D*	*Group E*
0.1	100	100	100	100	100
0.25	100	100	98	98	96
0.3	85	65	63	60	45
0.35	50	30	30	20	10
0.4	30	10	10	5	0
0.55	9	5	3	0	0
0.65	5	0	0	0	0
0.85	0	0	0	0	0

Discussion

The effects of GKS on PCV, Hb and RBC were dose-related; and were observed to be more intense at higher doses of GKS medication. The effect on Hb appeared to be the most sensitive to GKS and was observed at a dose (300mg/kg) which had no apparent effect on either PCV or RBC; GKS effects on PCV and RBC were manifested at a higher dose (600mg/kg). The phenomenon regulating this disparity in dose-response relationship regarding these erythropoietic indices is not well understood. There is a possibility that early interaction of GKS constituents with haematopoietic sites of action may activate haemoglobin production before influencing haematocyte preservation; in view of the fact that redox metabolism involving oxygen radicals regulates many physiological activities such as signaling, proliferation and apoptosis. The mitigation of these activities, by GKS, at diverse points or to varying degrees can introduce such disparity in PCV, Hb and RBC presentations. One established consequence of GKS medication is its antioxidant activity which complements the action of reduced glutathione produced via a metabolic pathway (pentose monophosphate shunt) in the erythrocyte. The essence of this antioxidant activity is the redox cascade which ultimately maintains the haeme iron in its ferrous state *vis-à-vis* the ferric state that is associated with production of defective methaemoglobin. The direct link between antioxidant activity and haemoglobin quality and/or quantity was shown by the observation that ascorbic acid supplementation, via its action as a free radical scavenger, increased significantly the haemoglobin levels in children suffering from sickle cell anaemia (Jaja *et al.*, 2002).

Whereas the erythropoietin modulated haemocyte lines showed increases in response to increasing GKS dosage, the white blood cells and platelets did not show any significant enhancement in numbers. This suggests that the GKS action relating to increase in blood cell lines may be effected through an influence on the stimulant cytokine erythropoietin. However, it should be noted that the peripheral blood leukocytes, as counted in this study, is only a small fraction of total body leukocyte pool. Additional leukocyte depots include the bone marrow and other body organs.

There was clearly a depreciation of the mean cell volume (MCV) with an increase in GKS dosage, implying that the cell size decreases significantly as dosage of medication was increased (from 300mg/kg to 900mg/kg and 1200mg/kg). This decrease can possibly be accounted for by the increase of mature erythrocytes relative to the larger immature forms (erythroblasts) due to the protective antioxidant action of GKS constituents (especially the biflavonoids like kolaviron). The interplay of protective actions, such as redox modulation of erythropoietin production, absence of anaemia or hypoxia and delayed release of reticulocytes into peripheral blood, ensures an aggregate decrease in MCV (Bunn, 1972). The decrease in MCV can also be attributed to possible functional deficiency of elemental iron, ostensibly facilitated by accelerated mobilization of iron stores, without concurrent iron supplementation in the diet. But the iron mobilization effect of ascorbic acid is defective, with a baseline value of less than 10% for hypochromic red cells (Sezer et al., 2002). This supports the observed significant

rise in MCHC at a dosage of only 300mg/kg; considering the decreases in MCH at doses of 300mg/kg, 900mg/kg and 1200mg/kg, signifying the consequence of depreciating iron stores. This depreciation was not low enough to reverse concurrent increases in PCV and Hb at the said doses of GKS.

GKS redication had no apparent effect on platelet counts; but there were significant dose-dependent increases in PT and APTT, compared to untreated controls. This is a remarkable reflection of anti aggregation ability typical of antioxidants. Aspirin was shown to decrease platelet activation *in vivo* during arterial thrombosis and cause a counterpart decrease in prothrombin activation *in vivo* (Ross *et al*., 1985). In the study herein presented, the increase in PT may be related to decreased platelet activation caused by antiaggregation effect of antioxidants in *Garcinia kola* seeds. However, some *in vivo* oxidants have been shown to increase prothrombin time, through another direct anticoagulant action. Stief *et al*., (2000) stated that blood coagulation factors I.V, VIII and X as well as platelets are sensitive to nonradical singlet oxygen –derived oxidants originating from activated polymorphonuclear leucocytes.

These chloramine – type oxidants were seen to act as anticoagulants in human plasma, prolonging PT, APTT and the thromboplastin time, at chloramine concentrations greater than 1mmol/l. In the absence of any pathological condition leading to observed increase in phagocyte activation in the animals used in this study, the increased PT and APTT values are attributable almost exclusively to the antiplatelet aggregation potentials of constituents in GKS, or their vitamin E-like prevention of oxidation of reduced vitamin K. Oxidation leads to carboxylation of vitamin K-dependent clotting factors, thus affecting their maximal functionality (Knight, 1995).

Serum cholesterol and triglyceride values were markedly decreased with increasing dosage of GKS. This observation is reminiscent of the action of some dietary isoflavones (eg., soy bean isoflavones) that have been observed to decrease the levels of total plasma lipids, total cholesterol and triglyceride (Yousef et al., 2004).

Chemical oxidative actions have been shown to exert haemolytic influence through lipid peroxidation of the red blood cell membrane and interference with metabolic activities that ensure the cell's integrity. Ascorbic acid, a known antioxidant, was shown to prevent oxidative damage of the cell membrane (Jaja *et al*., 2002); and vitamin C supplementation reduced erythrocyte lyses at 50% NaCl concentration, compared to controls in an osmotic fragiligram. These observations are similar to that reported in this study for GKS, showing that the antioxidant activities of constituents in *Garcinia kola* seeds can be protective against cell lysis.

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