JECA

Effects Of A Diet Containing Garcinia Kola Seeds On The Blood Of Adult Male Wistar Rats

[1]*E.A. ESOM , [2]F.C. AKPUAKA , [2]C.I.P.ANIBEZE

[1]Department of Anatomy, College of Medicine,University of Nigeria, Enugu Campus

[2]Department of Anatomy, College of Medicine and Health Sciences, Abia State University, Uturu.

*Author for correspondence

ABSTRACT

To study the effects of ingestion of G.kola diet on the blood profile of adult wistar rats, forty adult male wistar rats were used.They were obtained from the animal house of the Faculty of Pharmaceutical sciences, University of Nigeria Nsukka and divided into four (4) groups I-IV of ten (10) rats each. They were acclimatized to the conditions of the animal house of the University of Nigeria Teaching Hospital Enugu for a period of two (2) weeks. Groups I, II, and III were fed 30% w/w, 20%w/w and 10%w/w G.kola diet respectively while group IV served as control and was fed ad libitum with growers mash.

At the end of the experimental period of 10weeks the rats were starved overnight and their blood samples were collected in heparinised tubes. The blood samples were analyzed using routine laboratory methods. Fasting Blood Sugar (FBS), Packed Cell Volume (PCV), White Blood Cell (WBC) count (total and differential) were analyzed. There was a decrease in blood sugar as dose increases. The mean PCV and lymphocyte count increased as dose increases, while the eosinophils, neutrophils and monocytes decreased as dose increases. The values use subjected to statistical analysis.

The dose dependent decrease in blood glucose proved significant only between groups I,(30%w/w) and IV (control), when compared using student's t-test.The results were discussed.

Keywords: Medicinal plants. Biflavonoids. Hematological parameters

Garcinia kola (Heckel) is huge specie of spreading forest tree commonly found in the moist rainforest region distributed throughout West and Central Africa (Plowden, 1972, Ainslie, 1973; Keay et al, 1964). In Nigeria, it is found in the east and also in Ijebu Ode, Benin, Calabar and Ikom (Oliver, 1960, Keay et al, 1964).

It is commonly called 'bitter' kola because of its bitter taste and 'false' kola because of its use as a substitute to kolanuts.

Many workers have identified the seeds as medicinal against many diseases such as dysentery and diarrhea (Ainslie 1973), respiratory decongestion and relief of sour throat (Orie and Ekon, 1993) treatment of asthma (Ebomoyi, 1995), laryngitis and diabetes (Iwu et al 1990).

Ebana et al (1991), Aina and Uko (1991), Madubunyi (1995) and Akoachere et al (2002) reported its use as antimicrobial/antibacterial agents while Farombi et al (2000) demonstrated its use as antidotes to poison. Olajide (1999) reported that it could be antithrombotic. Biflavonoids have been implicated as active ingredients in the seeds of G. kola (Osisiogu 1964, Waterman and Hussain 1982). The wide medicinal uses ascribed to G.kola attracted many people to its regular consumption in large quantity as kola substitute, medicine, or junks and snacks.

The indiscriminate use of G. kola is not proper unless it proved to be entirely harmless to the body. Blood as a transport vehicle for substances in the body would carry the active ingredients of G.kola to the target organs as it achieves its medicinal actions.

The action of these substances on the blood itself needs to be studied so as to establish its safety or otherwise on the blood.

This study aims at investigating the effect of G.kola consumption on the blood profile of adult male albino rats.

MATERIALS AND METHODS

Procurement Of Animals

Forty (40) male Albino Rats were obtained from the animal house of the Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka. They were divided into four (4) groups I, II, III and IV with ten (10) rats in each group properly kept in cages with screened tops and

acclamatised to the conditions of the animal house of the College of Medicine, University of Nigeria Teaching Hospital, Enugu for a period of two (2) weeks. During the acclimatization period, they were fed ad libitum with growers mash produced by Top Feeds® Plc.

Preparation of g. Kola diet

G. kola seeds were purchased. Their seed coats were removed and the seeds were dried in the sun and ground to powder.

The powdered G. kola was mixed with the animal feed (Growers mash by Top Feeds® Plc) in various proportions to obtain 10%w/w, 20%w/w, and 30%w/w respectively using the following formula

$$\frac{\text{Mass of } \textit{G. kola} \text{ powder}}{\text{Mass of } \textit{G. kola} \text{ powder + mass of mash}} \times 100$$

Administration of g. Kola diet

Groups I, II and III respectively were fed with 30% w/w, 20% w/w, and 10% w/w G. kola diet. Group IV (control) was fed with normal, diet. Equal quantity of feed was given to all groups over the period.

At the end of the experimental period of ten (10) weeks, the feeds were withdrawn about 10pm. At the dawn of the next day, blood samples were obtained from the animals in heparinised tubes containing a dipotassiun salt of ethyl ditetra acetic acid (K_2EDTA) for FBC analysis and sodium fluoride for the blood sugar analysis.

Analysis Of Samples

With Mediguard Electronic Colorimeter, Glucose Oxidase method was used for theestimation of Fasting Blood Sugar (FBS).

Packed Cell Volume (PCV) was obtained using heparinised capillary tube and haematocrit centrifuge. For total White Blood Cell (WBC) count, 1% glacial acetic acid tinged with gentian violet was used. For platelet count, 1% ammonium xalate was used. Thin Blood films were obtained and stained with Leishman's stain for the differential WBC count and the films were studied under the light microscope.

RESULTS

There was a decrease in the blood sugar between the groups as the dose increases. The mean PCV values showed a dose dependent increase, which is not statistically significant (p 0.05). The basophils remained the same in all groups. The eosinophils and neutrophils decreased as the dose increases while the lymphocytes increased as the dose decreases. The monocytes increased between groups IV (control) and III (10%w/w) and then decreased as the dose increases. The lymphocytes increased as the dose increases. These are summarized in table1.

The values obtained were subjected to statistical analysis. Using Analysis Of Variance (ANOVA) to compare the groups the changes were not statistically significant (p 0.05) (Table 2). Using students t- test, each group was compared with the control. Only the Fasting Blood Sugar (FBS) of group I showed a statistically significant change when compared with the control (p 0.05) (Table 3).

DISCUSSION

The decrease in blood sugar as dose increases is interesting. Esom et al (2006) reported that ingestion of G. kola diet increases the storage of glycogen in the liver as the dose increases. Thus G. kola diet achieves an increase in liver glycogen and a decrease in blood sugar as the dose increases. The decrease in blood sugar and the converse increase in liver glycogen are in line with the principles of blood sugar regulation (Felig, 1975; van de Werve, 1987; Harris and Crabb, 1992; Fischbach, 1996, Stuart, 1999). In addition, the primary target at the treatment of diabetes is reduction of blood sugar. The reduction of blood sugar in the experimental groups agrees with the findings of Iwu et al (1990) on the antidiabetic uses of G. kola. Therefore, consumption of G. kola tends to decrease blood sugar and enhance glycogenesis.

The basophils are said to be closely associated with allergic reaction and substances that promote inflammation (Guyton and Hall, 2000; Stuart, 1999; Bannister, 1995). Already it

Table 1: Blood Profile (Mean Values) In Different Groups

Variable	Group I (N=10)	Group II (N=10)	Group III (N=10)	Group IV (N=10)
PCV (%)	39.00±1.05	38.70±1.06	37.60±2.50	37.60±2.22
Total WBC (mm^{-3})	5340.00 ± 697.93	5390.00 ±738.54	5370.00 ±678.31	5290.00 ± 574.36
Platelets (mm^{-3})	253700.00 ± 22090.97	256800.00 ±21373.92	250300.00 ±20542.91	244900.00 ± 16515.65
Basophils (%)	0.40±0.52	0.40±0.52	0.40±0.52	0.40± 0.52
Eosinophils (%)	0.60±0.70	0.70± 0.48	0.90±0.88	0.90±0.74
Neutrophils (%)	67.30±2.45	67.80±3.36	68.20±3.22	68.60±1.90
Monocytes (%)	0.90±0.88	1.00±0.82	1.10±0.88	0.80±0.63
Lymphocytes (%)	30.80±2.57	30.10±3.78	29.40±3.34	29.30±1.34
Fasting Blood Sugar ($mmolL^{-1}$)	3.790±0.233	3.91±0.074	3.92±0.215	3.98±0.140

Table 2: Comparison Of Mean Values Of The Blood Profile In The Different Groups By Analysis Of Variance (Anova)

Variable	F Test	Degrees of Freedom	p-value
PCV (%)	1.596	3	0.207
Total WBC (mm^{-3})	0.042	3	0.989
Platelets (mm^{-3})	0.634	3	0.598
Basophils (%)	0.000	3	1.000
Eosinophils (%)	0.443	3	0.724
Neutrophils (%)	0.395	3	0.757
Monocytes (%)	0.256	3	0.856
Lymphocytes (%)	0.574	3	0.636
Fasting Blood Sugar ($mmolL^{-1}$)	2.018	3	0.129

Table 3: Comparison Of Mean Values Of Blood Profile Of Different Groups With The Control Group Using Students T-Test

Variable	Group I (N = 10)	Group II (N = 10)	Group III (N = 10)
PCV (%)	t = 1.8	t = 1.41	t =0.00
	p = 0.088	p = 0.174	p = 1.000
Total WBC (mm^{-3})	t = 0.17	t = 0.34	t = 0.28
	p = 0.863	p = 0.739	p = 0.779
Platelets (mm^{-3})	t = 1.01	t = 1.39	t = 0.65
	p = 0.326	p = 0.181	p = 0.525
Basophils (%)	t = 0.00	t = 0.00	t = 0.00
	t = 1.000	t = 1.000	t = 1.000
Eosinophils (%)	t = 0.63	t = 0.00	t = 0.84
	p = 0.536	p = 1.00	p = 0.410
Neutrophils (%)	t = 0.27	t = 0.34	t = 0.70
	p = 0.789	p = 0.739	p = 0.491
Monocytes (%)	t = 0.26	t = 0.88	t = 0.51
	p = 0.796	p = 0.392	p = 0.617
Lymphocytes (%)	t = 0.44	t = 0.09	t = 1.05
	p = 0.666	p = 0.931	p = 0.307
Fasting Blood Sugar ($mmolL^{-1}$)	t = 2.21	t = 1.40	t = 0.74
	p = 0.040*	p = 0.179	p = 0.469

* Statistically significant

has been demonstrated that Kolaviron is anti-inflammatory (Ebomoyi, 1995; Olajide, 1999; Braide, 1990). Therefore, one would not expect to have increased basophils in the experimental groups since G. kola would not be expected to cause allergy and inflammation.

The granules in the eosinophils contain toxic chemicals, which are used to attack infecting organisms. In allergic disorders, the ratio of eosinophils rises (Bannister, 1995). The decrease in the percentage of eosinophils as the dose increases may be associated with the fact that the Flavonoids of G. kola seeds inhibits various agents of allergic disorders and worm infestations (Ebomoyi, 1995; Braide, 1999; Olajide, 1999; Lewis et al, 1977). Since these disorders may have been already forestalled by the presence of biflavonoids of G. kola seeds, it should be expected that the eosinophils should fall with increasing dose since there was little or no allergic- disorder- predisposing factors in the blood of the treated groups.

The neutrophils slightly decreased as dose increases. Neutrophils are phagocytes, which attack microbes. At higher doses, the percentages of neutrophils are lower. This could be attributed to the antimicrobial actions of biflavoniods of G. kola seeds (Ebana et al, 1991; Madubunyi, 1995), which may have cleared the blood of certain pathogens. Thus the neutrophils count would be low at higher doses since the flavonoid as antioxidants minimizes the incidence of cell damage.

Some monocytes are mobile (in the blood) while others are fixed (in the tissues) (Krause, 1996; Stuart, 1999). The monocytes showed changes in an irregular pattern, the cause of this is not strictly known. However, it is possible to attribute the variations to the different genetic constituents of the individuals in the groups with relation to the monocytes- macrophage system.

The lymphocytes circulate in blood to provide it with guards against foreign materials (immune system). The increase in lymphocyte count at higher doses may be explained in terms of the antimicrobial activity of the flavonoids of G. kola, which may help in activating T-lymphocytes. The platelets are responsible for blood clotting. In this study, they showed a dose dependent increase. The basophils which may secrete substances that promote bleeding (anti-coagulants) had earlier been shown to remain the same in all the groups. The increase in platelet population may suggest that G. kola enhances platelet count and thus may be capable of impeding excessive haemorrhage when cuts are incurred.

The PCV increased as dose increases. PCV is used to eliminate the erythrocyte content of blood (Stuart, 1999; Guyton 2000). Adaramoye and Akinloye (2000) demonstrated the protective effect of kolaviron against erthrocyte damage in rats.

The few crenated red blood cells observed in group I may not be seen as pathological. This is because Bessis (1973) reported that a few erythrocytes in normal blood assume a shrunken crenated form. These crenated cells may be erythrocytes whose destruction were delayed as a result of ingestion of G. kola seeds. Besides, the group recorded the highest PCV value.

The changes observed in the mean values of the total WBC and counts are not dose dependent. So they cannot be attributed to the consumption of G. kola. Rather, it may be associated with the specific constitution of the individual members of a particular group. However, none of the values was below the normal values.

From our findings, consumption of G. kola does not prove to be harmful to the blood cells. We can also conclude that consumption of G. kola reduces blood glucose.

REFERENCES

Adaramoye, OA; Akinloye, O (2000). Possible Protective Effect of Kolaviron on Carbon tetrachloride- induced Erythrocyte Damage in Rats. Biosci Rep. 20(4): 259-264.

Aina, JO and Uko, EE (1991). Preliminary studies on Tropical Hop Substitutes. Tropical science 31(4): 379-385.

Ainslie, JR (1973). List of plants used in Native Medicine. Imperial Forestry institute: 42.

Akoachere, JF; Ndig, RN; Chenwi, EB; Ndig, LM; Njock, TE and Among, DN(2002) Antibacterial Effect of Zingiber officinale and Garcinia kola on Respiratory Trait Pathogens. East. Afr. Med. J. 79(11): 588-592.

Bannister, LH (1995). The Blood in Williams, PL; Bannister, LH; Berry, MM et al (eds.). Grays Anatomy. 38th Edition. Churchill Livingstone, New York.

Bessis, M (1973). Living Blood cells and their ultrastructure. Springer, Berlin: 1401.

Braide, VB(1990). Pharmacologic effect of G.kola seeds in the Rat. Phytoterapia Research Vol. 4(1): 39-41.

Ebana, RUB; Madunagu, BE; Ekpe, ED and Otung, IN (1991). Microbiological Exploitation of Cardiac Glycosides and Alkaloids from G. kola, Borreria ocymodes, Kola nitida and Citrus aurantifolia. Journal of Applied Bacteriology 71(5): 398-401.

Ebomoyi, ME (1995). Effect of G. kola ingestion on Peak Expiratory Flow Rate. Nigeria Journal of Physiological Sciences vol. II (1/2): 27-28.

Farombi, OE; Tahteng JG, Agboola AO, Nwankwo JO, Emerole G. O (2000). Chemoprevention of 2-acetylaminoflurene- induced hepatotoxicity and lipid peroxidation in rats by kolaviron- a Garcinia kola seed extract. Food Chem. Toxicol 38(6): 535-541.

Esom, EA; Akpuaka, FC; and Anibeze, CIP (2006). Effects of a Diet containing G.kola Seeds on the Liver of Adult Male Albino rats. J. Expt. & Clinical Anatomy (In press)

Felig, P (1975). The Liver in Glucose Homeostasis in Normal Man and Diabetes. In: Valance- Owen J. (ed.) Diabetes. University Park Press, Baltimore: 378-391.

Fischbach, FT (1996). A Manual of Laboratory and Diagnostic Tests. 5th Edition. Lippincott-Raven Publishers, Philadelphia: 2308 -2315

Guyton, AC and Hall, EJ (2000). Textbook of Medical Physiology. 10th edition. WB Saunders, Philadelphia: 774-775; 798-802; 883-888; 973 -976.

Harris, RA and Crabb, DW (1992). Metabolic Interrelationships. In: Delvin, TM (ed). Textbook of Biochemistry with Clinical Correlations. 3rd edition. Wiley-Liss, New York: 575-601.

Iwu, MM; Igboko, OA; Okunji, CO; Tempesta, MS (1990). Antidiabetic and Aldose Reductase Activities of biflavonoids of G. kola. J. Pharm Pharmacol. 42(4): 290-292.

Keay, RWJ; Onochie, CFA and Stanfield, DF (1964). Nigerian Trees II. Department of Forestry Research. Ibadan: 135.

Krause, WJ (1996). Essentials of Human Histology 2nd Edition. Little, Brown and Company Inc., New York: 589-593

Lewis, WH et al (1977). Medical Botany: Plants Affecting Man's Health. John Wiley; New York: 1-232.

Madubunyi, II (1995). Antimicrobial Activities of the Constituent of G. kola seeds. International Journal of Pharmacognosy 33(3): 232-237.

Olajide, OA (1999). Investigation of the Effects of selected Medicinal plants on Experimental thrombosis. Phytotherapia Research 13(3): 231-232.

Oliver B (1960). Medicinal Plants in Nigeria. College of Arts and Technology (Lectures) Ibadan.

Orie, NN and Ekon, EUA (1993). Bronchodilator Effect of G. Kola. East African Medical Journal Vol. 7(3): 143-145.

Osisiogu, IGW (1964). A Preliminary Thin Layer Chromatographic study of the seed Extracts of G. Kola. India Current Science 33:552-553.

Plowden, CC (1972). A Manual of Plant Names. 3rd Edition. George Ltd, London: 239.
Stuart, IF (1999). Human Physiology. 6th edition. McGraw-Hill Inc, Boston: 580-602.

Van de Werve, G and Jeanrenaud, B (1987).Liver Glycogen Metabolism: an overview. Diabetes Metabolism Rev. 3:47.

Waterman, PG and Hussain, RA (1982). Major Xanthones from G. quadrifaria and G. staudtii stem Barks. Phytochemistry 21(8): 2099-2101.