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Modulatory effects of the seed extracts and fractions of *Garcinia kola* Heckel (Clusiaceae) on the immune responses

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ABSTRACT

Garcinia kola has versatile application in folk medicine and is believed to increase immunity and general resistance to infections, but there are no scientific data on the effect of *G.kola* on the immune system. In this study, the immunomodulatory activities of the methanol seed extract of *G.kola* seed (ME) and its fractions were studied on some antigen-specific and non-antigen specific components of the immune system such as the delayed-type hypersensitivity response (DTHR), primary and secondary humoral responses, phagocytic activity of polymorphonuclear neutrophils (PMNs), haemolytic activity of complement, and *in vivo* leucocytes mobilization. The methanol extract, ME (250 and 500mg/kg) produced a dose-related and significant (P<0.05) inhibition of DTHR induced by Sheep Red Blood Cells (SRBCs) in rats by 44 and 68%, respectively. DTHR was also significantly (P<0.05) inhibited by EF (250 and 500 mg/kg) and CF (500 mg/kg) by 52, 64 and 24%, respectively. Bioactivity study on the column fractions shows that the flavonoid-rich fraction, F_3 exhibited the highest inhibitory activity of DTHR of 46.15%. Sheep erythrocytes-specific antibody synthesis, especially the secondary humoral response was significantly (P < 0.05) increased by ME (250 and 500 mg/kg) and EF (500 mg/kg) by 26.09, 34.74 and 21.74%, respectively. At a dose of 500 mg/kg, ME and EF caused an increase in total leucocytes mobilization up to 62.66 and 17.72% into the peritoneal perfusates when compared to the untreated group. The ME and column fraction, F_3 also showed significant (P<0.05) inhibition of complement-induced haemolysis of SRBC up to 26.27 and 29.66%, respectively. The results of this study establish cellular and humoral immunomodulatory properties of *G.kola* extract and justify its inclusion as immune adjuvant in dietary supplements.

KEYWORDS

Garcinia kola;
Complement activity;
Delayed type hypersensitivity;
Immunomodulation;
Humoral response;
Leucocytes mobilization.

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INTRODUCTION

The body has an elaborate and intricate defence system which counters the effects of microorganisms, pathogens and other foreign substances which invade the system. The human immune system consists of two categories of defence mechanisms- the non-specific (innate) and the specific (adaptive) systems[1] The innate and adaptive immune defences could be modified by substances to either enhance or suppress the responses of these systems to pathogens and to other antigens[2]. Substances which are able to stimulate or suppress the immunity are both relevant in clinical practice.

The role of immunomodulating substances from natural sources in human disease prevention and treatment is increasingly being appreciated in this age of emerging new, chronic, and more virulent viruses. The search for such substances and lead molecules with these activities is currently a field of major interest all over the world[3] .There has been a growing interest in identifying and characterizing these natural compounds with immunomodulatory activities[4-9]. In response to infections or harsh growing environment, plants are known to produce a vast number of secondary metabolites that have demonstrated immunomodulatory and other related properties[10]. *Garcinia kola* Heckel (Clusiaceae) is a plant of versatile folk application and is claimed to posses a number of ethnomedicinal uses including, anti-inflammatory[11], antidiabetic[12], hypolipidaemic[13], antioxidant[14, 15], antimicrobial[16], antihepatotoxic[17], antiviral[18], and adaptogenic properties[19]. *Garcinia Kola* is a medium sized tree found in moist forest and widely distributed throughout West and Central Africa. The plant is highly valued in these countries for its edible nuts[20]. The seed, commonly known as bitter kola, is a masticatory agent and is a major kola substitute offered to guests at home and shared at social ceremonies.

Most of the pharmacological activities of *G.kola* are believed to be related to its ability to increase immunity and to its antioxidant properties[21]. Hitherto, there is lack of scientific data on the effects of *Garcinia kola* on the components of immune system, some herbal products have the plant extract included as immune-boosting agent[22]. Locally, *G.kola* seed is chewed by people living with HIV in the belief that it boosts the immune system. The present study is aimed at investigating the effects of *G.kola* seed extract and its fractions on the specific and non-specific components of the immune system.

EXPERIMENTALS

Preparation and extraction of plant material

Seeds of *Garcinia kola* Heckel (Clusiaceae) were obtained commercially from a local market-"Oba Nsukka", Enugu State, Nigeria in the month of June, 2005. The plant material was authenticated by Mr A.Ozioko, a plant taxonomist of the International Centre for Ethnomedicine and Drug Development (Inter CEDD), Nsukka. The seeds were peeled, dried and pulverised. The *G.kola* seed powder (2 kg) was extracted with methanol in a soxhlet extractor for 24 h and concentrated in a rotary evaporator to obtain 487.2g (24.36%, w/w) of the methanol extract (ME). A portion of ME (200g) was successively and exhaustively extracted with petroleum ether (60-80^{0}C), chloroform, ethyl acetate, and methanol in that order of increasing solvent polarity. Concentrating the solutions under reduced pressure afforded 19.86g of petroleum ether soluble fraction (PF), 40.48g of chloroform soluble fraction (CF), 103.78g of ethyl acetate soluble fraction (EF), and 23.02g of methanol soluble fraction (MF). The extracts and fractions were subjected to phytochemical analysis using the procedures outlined by Harbourne (1998)[23].

Chemicals and reagents

Pyrogen-free sterile normal saline, phosphate buffer saline (NaCl- 8g, KCl-0.2g, Na_2PO_4-1.44g, KH_2PO_4-0.24 in 1 L distilled water, pH-7.4), Veronal buffer saline (NaCl-42.5g, diethylbarbituric acid-2.875g, sodium barbiturate-1.0g, $MgCl.6H_20$ -0.84g, CaCl-0.14g, and distilled water to 1L, pH-7.2), Nitro blue tetrazolium (NBT) procured from Sigma chemical Co. (St. Louis, MO, USA). All reagents and solvents were of analytical grade.

Animals

Adult rats of Wistar strain (170-200g) and adult Swiss albino mice (20-28g) of both sexes obtained from the Animal House of the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka were

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used in the study. The animals were housed under standard conditions (25±2^{0}C and 12-h light/dark cycle). They were fed with standard pellets and allowed free access to drinking water. The use and care of laboratory animals were conducted in accordance with the internationally accepted principles as found in the European Community guidelines (EEC Directive of 1986; 86/609/EEC).

Antigen

Fresh blood was obtained from a male sheep in the animal farm of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The sheep red blood cells (SRBCs) were washed four times in a large volume of pyrogen-free sterile normal saline by centrifugation at 3000×g for 10min on each occasion and thereafter adjusted to a concentration of 10^9 cells/ml for immunization and challenge.

Bioactivity-guided fractionation of crude extract

The methanol extract (ME) and the fractions of *G.kola* was screened for immunomodulatory activities. Cell-mediated delayed type hypersensitivity response (DTHR) induced by sheep red blood cells (SRBCs) in the hind paw of rats was used as activity guide in the fractionation of ME[3]. Firstly, the methanol extract which had shown significant inhibition of DTHR in a prior pilot study was partitioned in solvents of graded polarity; n-hexane (HF), chloroform (CF), ethyl acetate (EF), and methanol (MF) and the solvent fractions obtained were screened for immunomodulatory activities. The ethyl acetate soluble fraction (EF) which showed highest inhibition of DTHR was subjected to further studies and fractionation in a chromatographic column.

Column chromatographic separation of ethyl acetate fraction (EF)

The ethyl acetate soluble fraction of *G.kola* (EF) was subjected to column chromatographic separation since it showed the highest inhibition of DTHR in the bioactivity-guided studies. The EF (20g) was separated in a dry-packed silica gel column of width, 4cm and length, 40cm. The extract was mixed with the silica gel (70-230 mesh, Merck Germany) and loaded on the top of pre-packed column. The column was successively eluted with gradient mixtures of petroleum ether: ethyl acetate (4:6, 3:7, 2:8, 1:9) and ethyl acetate: methanol (I: 0, 1: 1 and 0: 1). These solvent systems were chosen on the basis of a prior empirical analytical TLC elution of spots of EF on a pre-coated Silica gel GF 254 coated foils (Uniplate™, Analtech Co., Delaware, USA). A total of 215 aliquots of 15 ml were collected in serially arranged test tubes. The various aliquots were pooled into six column fractions (F_1-F_6) using phytochemical tests and UV absorption spectra as guide. The pooled column fractions were subjected to phytochemical studies using methods of Harborne (1998)[23].

The immunomodulatory effects of the column fractions (F_1-F_6) on DTHR and primary humoral antibody response were studied. Fraction F_3 which showed the highest inhibition of DTHR was also studied further for other immunomodulatory properties.

Delayed type hypersensitivity response (DTHR)

Delayed type sensitivity was induced in rats using sheep red blood cells (SRBC) as antigen. The rats placed randomly into groups of 5 animals were sensitized by subcutaneous injection of 0.1 ml of 10^9 cells/ml SRBC (day 0) in the plantar region of right hind foot paw and challenged on the 5th day by subcutaneous injection of the same amount of antigen into the left hind paw. The oedema produced by antigenic challenge in the left hind paw was measured as the difference in volume of water displaced by the paw before and 24 h after the challenge. *G.kola* extract, ME (100, 250 and 500mg/kg), the solvent fractions (250 and 500mg/kg) and the column fractions of EF (100mg/kg) were administered orally from 3 days prior to sensitization and continued once daily till the challenge.

Humoral antibody (HA) synthesis

Rats were immunized by an intraperitoneal injection of 0.2ml of 10^9 SRBC/ml on day 0 and challenged by injecting the same amount (i.p.) on day 7. Primary antibody titre was determined on day 7 (before the challenge) and secondary titre on day 14[24] by the haemag glutination technique[25]. *G.kola* extract, ME (100, 250 and 500 mg/kg), the solvent fractions (250 and 500 mg/kg) and the column fractions of EF (100 mg/kg) were administered (p.o.) 3 days prior to sensitization and continued once daily for 7 days post-challenge. Blood samples were obtained from retro-orbital plexus

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and 25µL serum was serially diluted two-fold in 96-U well microtitre plates using pyrogen-free sterile normal saline. The diluted sera were challenged with 25µL of 1% (v/v) SRBC and then incubated at 37^0C for 1 h. The highest dilution showing visible haemagglutination was taken as antibody titre. Antibody titres were expressed in graded manner, the minimum dilution (1/2) being ranked as 1 (Calculated as -Log_2 of the dilution factor). The mean ranks of different treatment groups were compared for statistical significance using one way analysis of variance (ANOVA).

In vivo leucocytes mobilisation

The effect of the extract and fractions on the in *vivo* leucocytes migration induced by inflammatory stimulus was investigated using the methods of Ribeiro et al.[26]. One hour after oral administration of ME (100, 250 and 500mg/kg) and the solvent fractions (250 and 500mg/kg), each rat in the groups (n = 5) received intraperitoneal injections of 1 ml of 3%, w/v agar suspension in normal saline. Four hours later, the animals were sacrificed and the peritoneum washed with 5ml of a 5% solution of EDTA in PBS. The peritoneal fluid was recovered and total and differential leucocytes counts (TLC and DLC) performed on the perfusates.

In vitro immunostimulant activity studies by slide method.

In order to study the effect of treatment of *G.kola* extracts on phagocytic function of polymorphonuclear neutrophils (PMN), the *in vitro* slide culture technique was used[27,28].

Preparation of *Candida albicans* suspension

*Candida albican*s culture was incubated in Sabouraud dextrose broth overnight and then centrifuged to form a cell button at the bottom of the test tube. The supernatant was discarded and the cell button was washed 3-4 times with sterile phosphate buffered saline (PBS) and then centrifuged again. The washed cell button was re-suspended in a mixture of phosphate buffered saline and rat serum in proportion of 4:1. The count of *C.albicans* was adjusted to 10^8 cells/ml using a 0.5 McFarland standard.

Preparation of slide

About 0.2 ml of rat blood was smeared on a sterile glass slide and incubated at 37°C for 20 min to allow clotting. The slide was thereafter drained slowly with sterile normal saline, taking care not to wash the adhered neutrophils. The slide consisting of PMNs was flooded with predetermined concentrations of ME and fractions and then incubated at 37°C for 15 min. The PMNs were covered with *C. albicans* suspension and incubated at 37°C for 1 h. The slide was drained, fixed with methanol and stained with Giemsa stain.

Evaluation of phagocytosis

The slide was observed under 100x oil immersion objective and phagocytosis evaluated by the method previously described[28]. The number of *C.albicans* cells phagocytosed by PMNs on the slide was determined microscopically for 100 granulocytes using morphological criteria. This number was regarded as phagocytic index (PI) and was compared with PI of control treatment. This procedure was repeated for different concentrations (100, 50, 25, 12.5. 6.25µg/ml) of the various test samples. Immunostimulation was calculated using the following equation:

$$\text{Stimulation of phagocytosis (\%)} = \frac{\text{PI(test)} - \text{PI(control)}}{\text{PI(control)}} * 100$$

Studies on the haemolytic activity of complement

1. Sensitization of the antigen

The SRBC (4×10^8 cells/ml), used as indicator for the complement classical pathway, was sensitized by incubating at 37^0C for 30min with an equal volume of subhaemaglutination titre (1/100 dilution) of rat anti-sheep erythrocyte antiserum generated in-house according to previously reported method[29].

2. Complement assay

The effect of the extracts on the haemolytic activity of complement system through the classical pathway was investigated *in vitro* by a modified version of a microtitre assay[24]. Briefly, a stock solution of ME, EF and F_3 were prepared in DMSO and then serially diluted 2-fold in 0.5ml of Veronal buffer saline (VBS^{++})[containing (in g/L) NaCl-42.5, diethyl barbituric acid-2.875, sodium barbiturate-1.0, $MgCl_2.6H_2O$-0.84, $CaCl_2$-0.14; pH-7.2]. Four different concentrations (400, 200, 100 and 50µg/ml) of each sample obtained in triplicate test tubes were used for

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the test. Thereafter, 0.5ml of 1:20 dilution of fresh guinea pig pooled serum (GPS) in VBS^{++} was added to each tube. After preincubation at 37^0C for 30 min, 0.5 ml of the suspension of 4×10^8 sensitized SRBC was added. The tubes were incubated at 37°C for 1 h and the reaction stopped by centrifugation at 1500×g for 5min. A 0.5 ml aliquot was drawn from each tube and mixed with 2ml of distilled water and the degree of haemolysis was measured spectrophotometrically at 541nm. Controls in this assay consisted of similarly treated incubates in which samples (0% stimulation) or guinea pig serum (0% lyses) were omitted.

The effect of the treatments on the haemolytic activity of complement was calculated by the relationship:

$$\text{Inhibition of complement (\%)} = \frac{\text{Asborbance(control)} - \text{Absorbance(test)}}{\text{Absorbance(control)}} \times 100$$

Statistical analysis

Results were analysed using one way Analysis of Variance (ANOVA) followed by Fischer Least Square Difference post hoc test and then expressed as mean ± S.E.M. Differences between means of treated and control groups were considered significant at $P < 0.05$.

RESULTS

The phytochemical tests on ME showed positive reaction for sterol, terpenes, glycosides, flavonoids, saponins, starch and sugars. The extractive yield and the occurrence of the phytoconstituents in the different fractions are shown in TABLE 1.

The extract, ME (250 and 500 mg/kg) administered orally for 7 days produced a dose-related and significant (P<0.05) inhibition of DTHR induced by SRBCs in rats by 44 and 68%, respectively. DTHR was also significantly (P<0.05) inhibited by EF (250 and 500 mg/kg) and CF (500mg/kg) by 52, 64 and 24%, respectively (TABLE 2). The other solvent fractions did not produce significant (P>0.05) inhibitions of DTHR. Bioactivity study on the column fractions shows that F_3 exhibited the highest inhibitory activity on sheep erythrocytes-induced DTHR (TABLE 3).

Administration of the ME and EF also increased the humoral antibody synthesis in the rats. The primary antibody titre was significantly (P>0.05) increased by

TABLE 1: The yield and phytochemical constituents of *G.kola* extract and fractions

Extract/ Fraction	Yield*	Phytochemical constituents
ME	487.2 g (24.36 %)	Flavonoids, Glycosides, saponins, starch, sugars, sterols, triterpenoids, tannins
PF	19.86 g (9.93 %)	sterols, and triterpenoids
CF	40.48 g (20.24 %)	Flavonoids, Glycosides, and starch
EF	103.78 g (51.89 %)	Flavonoids, Glycosides, saponins, and starch,
MF	23.02 g (11.51 %)	Flavonoids, Glycosides, saponins, starch, sugars, and tannins
F_3	7.94 g (39.7 %)	Flavonoids and traces of sterol and triterpenoids

*Percentage yield of the starting material is shown in paren thesis

TABLE 2: Effect of *G.kola* seed extract and solvent fractions on SRBC-induced delayed Type Hypersensitivity (DTH) reaction in rats

Treatment	Dose mg/kg, p.o.	DTH response Oedema (cm^3) (Mean±SEM)	Inhibition (%)
ME	100	0.21 ± 0.019	16.00
	250	0.14 ± 0.010	44.00**
	500	0.08 ± 0.012	68.00**
PF	250	0.22 ± 0.020	12.00
	500	0.24 ± 0.019	4.00
CF	250	0.21 ± 0.019	16.00
	500	0.19 ± 0.019	24.00*
EF	250	0.12±0.012	52.00**
	500	0.09 ± 0.019	64.00**
MF	250	0.22 ± 0.025	12.00
	500	0.25 ± 0.022	0.00
Levamisol	25	0.24±0.019	4.00
Control (Vehicle)	-	0.25± 0.016	-

N=5; * P < 0.05; ** P < 0.01

TABLE 3: The effects of the column fractions of EF on delayed type hypersensitivity and primary antibody synthesis in rats

Column fraction	Dose mg/kg, p.o.	DTH response (Mean oedema ± SEM) (cm^3) (% inhibition in parenthesis)	Primary humoral response (Mean titre± SEM)
F_1	100	0.53±0.04 (-1.92)	6.6±0.2 (3.12)
F_2	100	0.33±0.026* (36.54)	5.8±0.37(-9.38)
F_3	100	0.28±0.026* (46.15)	6.6±0.25 (3.13)
F_4	100	0.49±0.033 (5.77)	6.2±0.37 (-3.13)
F_5	100	0.50±0.044 (3.85)	6.2±0.37 (-3.13)
F_6	100	0.45±0.02 (13.46)	5.6±0.40 (-12.5)
Negative control	-	0.52±0.026	6.4±0.25

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kg) when compared to the untreated group (TABLE 4). The column fractions did not show remarkable effect on the primary antibody synthesis in rats (TABLE 3).

In the *in vivo* leucocytes migration studies, ME (100, 250 and 500mg/kg) caused a significant (P<0.05) and dose-related increase in leucocytes mobilization into the peritoneal perfusates when compared to the control group. The EF at 500mg/kg also caused a similar increase in leucocytes mobilization. The proportion of neutrophils in the mobilized leucocytes was higher than the lymphocytes (TABLE 5).

The extract, ME and the fractions EF and F_3 did not produce any significant (*P*>0.05) effect on the phagocytic activity of PMNs at the concentration range of 6.25-100µg/ml applied (TABLE 6).

The methanol extract (200 and 400µg/ml) and fraction F_3 (200 and 400µg/ml) caused a significant (*P*<0.05) inhibition of complement-induced haemolysis of SRBC in a concentration-dependent manner. Although, the EF caused a similar inhibition of haemolytic activity of complement, the values were not significant over the concentration range of 50 and 400µg/ml tested (TABLE 7).

TABLE 4: Effect of ME and EF on SRBC-induced humoral antibody titre (HA) in rats

Treatment	Dose mg/kg, p.o.	Haemagglutination antibody titre¶	
		Primary	Secondary
ME	100	4.2±0.20 (10.53)	5.0±0.316 (8.70)
	250	4.4±0.25* (15.79)	5.8±0.20* (26.09)
	500	4.4±0.51* (15.79)	6.2±0.20** (34.74)
EF	250	4.6±0.40 (21.05)	5.0±0.45 (8.7)
	500	5.0±0.32*(31.58)	5.6±0.240* (21.74)
Levamisol	25	4.2±0.49(10.53)	5.2±0.374*(13.04)
Control (Vehicle)	-	3.8±0.374	4.6±0.245

¶Values are calculated as Log_2 of the highest dilution showing visible agglutination and expressed as mean±SEM of 5 animals in each group. Percentage humoral stimulation is shown in parenthesis.* *P*<0.05; ** *P*<0.01

TABLE 6: Effect of *G.kola* seed extract and fractions on phagocytic activity of polymorphonuclear leucocytes

Extract	Concentration (µg/ml)	Phagocytic Index (PI)	Stimulation of phagocytosis (%)
ME	6.25	206.7±11.54	6.90 NS
	12.5	216.7±3.33	12.07 NS
	25	236.7±5.77	22.41 NS
	50	196.67±12.01	1.72 NS
	100	183.3±6.67	-5.17 NS
EF	6.25	203.3±14.53	5.17 NS
	12.5	210.0±11.55	8.62 NS
	25	213.3±8.82	3.44 NS
	50	216.0±3.33	12.07 NS
	100	223.33±8.82	15.52 NS
F_3	6.25	202.23±2.97	4.62 NS
	12.5	185.86±3.93	-3.85 NS
	25	196.28±10.30	1.54 NS
	50	193.3±7.87	0.00 NS
	100	200.72±2.58	3.84 NS
Control		193.3±8.82	-

N =3; NS = not significant (*P*>0.05); negative sign shows inhibition

TABLE 7: The effect of *G.kola* seed extract and fractions on haemolytic activity of complement

Extract	Concentration (µg/ml)	Absorbance (541 nm)	Inhibition (%)
ME	50.0	0.110±0.006	6.78 NS
	100.0	0.108±0.003	8.47 NS
	200.0	0.090±0.001	23.73*
	400.0	0.087±0.004	26.27*
EF	50.0	0.120±0.001	-1.69 NS
	100.0	0.113±0.002	4.24 NS
	200.0	0.101 ±0.001	14.41 NS
	400.0	0.098±0.005	16.95 NS
F_3	50.0	0.100±0.003	15.25 NS
	100.0	0.103±0.004	12.71 NS
	200.0	0.092±0.004	22.03*
	400.0	0.083±0.004	29.66*
Control (GPS alone, 0% inhibition)	-	0.118±0.001	-

**P*<0.05, NS = not significant (*P*>0.05) values are expressed as mean±S.E.M. (n = 3). All absorbance values were corrected for the mean absorbance (0.02±0.001) recorded for the 0% lyses control treatment. GPS = guinea pig pooled serum

TABLE 5: The effect of ME and EF on *in vivo* leucocytes migration in rats

Treatment	Dose (mg/kg)	TLC Mean±SEM (cells/mm^3) % increase in parenthesis	Differential Leucocytes counts (DLC) Mean±SEM (%)			
			Neutrophils	Lymphocytes	Monocytes	Eosinophils
ME	100	3600±70.7 (13.92)	64.4±1.69	35.2±1.88	0.2±0.2	-
	250	4080±233.2 (29.11)**	62.2±1.24	37.6±1.12	0.2±0.2	-
	500	5140±77.6 (62.66)**	65.6±1.69	34.4±1.69	-	-
EF	250	2300±70.7 (-27.21)	63.0±2.1	36.4±2.27	0.6±0.4	-
	500	3720±86.02 (17.72)*	61.2±2.58	38.6±2.46	0.2±0.45	-
Control	-	3160±248.2	58.6±0.60	39.0±2.32	0.4±0.4	-

Total Leucocytes count (TLC); N = 5; * *P*<0.05; ** *P*<0.01

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DISCUSSION

The control of disease by immunological means has two aspects, namely the development and improvement of protective immune status of an organism and the avoidance of undesired immunological side reactions. In several previous studies, medicinal plants extracts and secondary metabolites of natural sources have shown great potentials in modulating the immune system. In this study, we evaluated the effects of the methanol extract and fractions of *G.kola* on the different aspects of immunity. The extract and the various solvent and column fractions of *G.kola* exhibited different immunomodulatory activities on the different components of immune system investigated in this study.

The methanol extract, ME, the ethyl acetate fraction, EF and the column fraction, F_3 inhibited the manifestation of SRBC-induced delayed type hypersensitivity response (DTHR) in rats. DTHR study assesses the effect of the treatment on cell-mediated immune response which is usually initiated by actively sensitized T lymphocytes and is expressed locally by cellular infiltration and inflammation[30]. In DTHR, the lymphokines released by sensitized T cells contribute to the overall tissue damage and also recruit macrophages which release lysosomal products and enzymes thereby causing further tissue damage and thus chronic inflammation[31]. Although the reproduction of sensitized cytotoxic T cells takes some time to appear in the blood stream, it plays a central role in the manifestation of DTH reaction[32]. It is not certain if the observed inhibition of DTHR is related to the mechanisms for the anti-inflammatory activity of *G.kola* extracts reported in previous studies[11].

The humoral specific immune response was also significantly (P<0.05) increased by treatment with ME and EF, but not by F_3. The effect of treatments with the extracts and fractions on sheep erythrocyte-specific haemagglutination antibody titre in rats was investigated. A significant elevation of circulating antibody titre in the treated groups was recorded. The increase in antibody production recorded was more pronounced for the secondary humoral response with the booster dose of the antigen. This activity of the seed extracts is very important considering the role of the various antibody isotypes on immunological protection against pathogens and "foreign" substances. It also make the extracts from the plant potentially useful as vaccine adjuvants. At least three major cell types (the macrophages, B-lymphocytes, and T-lymphocytes) corporate in the process of antibody synthesis[33]. It is possible that the extract of *G.kola* act on one or more of these cells types to enhance antibody synthesis.

The stimulation of antibody production by *G.kola* extract and fractions is also very remarkable for so many reasons. The augmentation of immunoglobulins production could be beneficial in checking the establishment of infections by its involvement in different immune pathways which includes phagocytosis, neutralization of antigen, agglutination of antigen, complement fixation and cell lysis[1].

Treatment with ME and EF also increased agar-induced leucocytes mobilization into the peritoneum. This activity may help increase the general resistance and response of the body against microbial infections. The polymorphonuclear neutrophils, which engulf and eliminate invading microorganism, were the predominant cells in the peritoneal perfusates. It is known that the chemotactic movement of neutrophils towards the foreign body is the first and the most important step in phagocytosis[28].

The ingestion of microorganisms after coming in contact with neutrophils, studied by slide method, provides a rapid and simple means of assessing the overall phagocytic process[27,28]. Application of the extract and fractions produce a stimulation of phagocytic activity of PMNs which was largely non significant (P=0.05) when compared with the control treatment. The complement system, one of the major effectors mechanisms plays an important role in the amplification of specific immune responses, which are initiated by reaction of antibody or sensitized T-lymphocytes with the recall antigen. The activated complement components mediate a variety of tissue responses including inflammatory reactions[24]. The extract and fractions were incubated with guinea pig pooled-serum (GPS) at a concentrations range of 50-400µg/ml. The methanol extract caused a concentration-related inhibition of the haemolytic activity of complement as indicated by the higher absorbance values recorded for the haemolysed SRBC solution which was significant at concentrations above 200µg/ml. Similar inhibition was also shown by the EF and F_3. Apart from the formation of membrane attack complex which

lyses and destroys invading cells, complement fragments that are librated into the surrounding fluid have a number of other effects. These include chemotaxis, opsonisation and the stimulation of histamine release from mast cells and basophils[34]. Non-specific inhibition of complement activity is crucial in the modulation of hyper-deleterious immune reactions.

From our phytochemical studies, the methanol extract of *G.kola* contains flavonoids, saponins, glycosides, sterols, triterpenoids, and sugars. Also, a complex mixture of biflavonoids, prenylated benzophenones, xanthones and phenolic compounds have been reported[14,16,35,36]. Although we are yet to associate the effects of *G.kola* extract on immunity to specific constituent(s), a number of substances, including flavonoids, polysaccharides, terpenoidal glycosides and alkaloids have been reported to have either stimulatory or inhibitory effect on the various components of immune system[4,5,7,24]. In this study, all the immunoactive extracts and fractions of the *G.kola* consistently showed the presence of flavonoidal phytoconstituent.

This study also revealed that the *G.kola* seed extract and its fractions possess ambivalent immuno-activity behaviour since they were shown to possess either inhibitory or stimulatory effects on the components of the immune system depending on the type of immune protection, the mechanism and the cell type that is involved. All of these properties of *G.kola* extracts are clinically relevant and could be harnessed to regulate normal immune function.

CONCLUSIONS

The findings of this study establish cellular and humoral immunomodulatory properties of *G.kola* extract. The inclusion of *G.kola* seed as immune adjuvant in proprietary dietary supplement and herbal tonics could therefore be justified.

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