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Immunomodulatory activity of the total crude extracts of *Solanum inguivi* in the Wistar albino rats

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ABSTRACT

The immune system is the defense mechanism of the body and currently a number of disease conditions are on increase worldwide that either exaggerate or suppress it. The study investigated the immunomodulatory activity of the total crude extracts of *S. anguivi (indicum)* in Wister albino rats. Four groups each with 6 healthy adult animals of either sex weighting between 80 to 150g were used. Group I was dosed each with 1mL intragastrically of normal saline. Groups II, III and IV were each given 1mL of total crude extract of 125 mg/Kg bwt, 250 mg/Kg bwt and 500mg/Kg bwt respectively daily for 14 days. On the 15th day, whole blood was collected by puncturing the retro orbital vein of the rats. The RBC, immune blood cell count, HA titer and DTH were determined. Increasing the dose of the extract from 125 to 500mg/Kg bwt caused a reduction in the mean WBC count. The reduction at a dose of 500mg/Kg bwt was statistically significant ($p \leq 0.05$) as compared to the control group. The mean RBC count was higher than for the control group for all the doses but was not statistically significant ($p \geq 0.05$). The mean % lymphocytes were higher for all the doses than the control group but with no statistical difference between the 2 groups ($p \geq 0.05$) though however, there was a dose-dependent reduction in the mean % lymphocytes. The mean % lymphocytes were higher for all the doses than the control group but with no statistical difference between the 2 groups ($p \geq 0.05$) though however, there was a dose-dependent reduction in the mean % lymphocytes. The HA titer was higher than the control group and was statistically significant ($p \leq 0.05$) at 125 and 250mg/ Kg bwt as compared to the control group. The neutrophil adhesion were higher than the control group but were not statistically significant. The DHT were higher than for the control group and was statistically significant ($p \geq 0.05$) at a dose of 500mg/Kg bwt after 12 hours and 125mg/Kg bwt after 24 hours. The results show that the total crude leaf extracts of *S. anguivi* contained compounds with immunomodulatory activity.

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KEYWORDS

Immune system;
Immunomodulation;
S.anguivi;
Extracts.

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INTRODUCTION

The immune system is the defense mechanisms of the body and responds to the foreign bodies by two major mechanisms including the humoral and cell-mediated immune responses. Humoral immunity responds by antibody dependent and are produced after the B-lymphocytes have transformed into plasma cells that are responsible for secreting antibodies[1-6]. The antibodies are freely circulating in the blood (plasma) and other body fluid compartments. The cell-mediated immunity involves the activation of cellular immune cells such as leucocytes, macrophages, natural killer cells (NK), antigen-specific cytotoxic T-lymphocytes, and the release of various cytokines in response to an antigen[7,8]. The response of the immune system may at times be either exaggerated (immunostimulation) or suppressed (immunosuppression). Immunostimulation primarily involves stimulation of the non-specific immune system like the granulocytes, macrophages, complement and certain T-lymphocytes by the microbial infections, parasitic infection, environmental factors, chemicals and drugs that may lead to hypersensitivity reactions[4,9]. The immunosuppression may result from viral infections such as HIV, bacterial infections, stress as well as environmental or chemotherapeutic agents[10]. In some cases, activation of the immune system may lead to hypersensitivity reactions. Hypersensitivity reactions leads to undesirable damaging, discomfort that may sometimes lead to fatal body reactions that are classified according to Coombs and Gell (1975)[11], into four different types: type I, type II, type III and type IV, based on the mechanisms involved and time taken for the reaction to occur[12-14]. Frequently, a particular clinical condition (disease) may involve more than one type of reaction with the immunoglobulin or antibodies and immune cells involved[5,12,15]. Immunoglobulins are divided into 5 classes- IgM, IgG, IgA, IgE, IgD that can be distinguished biochemically and functionally[5,12,15].

However, the immune system is regulated by immunomodulation by various immunomodulator agents that can be a protein such as vaccines, microorganisms, a drug or chemical or herb[4,7,16,17]. Modulation of the immune responses to alleviate diseases has been of interest to many health workers and general population worldwide for many years[9,10]. Currently there is increased scientific interest in agents that can modulate the immune system in severely immunocompromised individuals like in cases of HIV, stress, malnutrition and many others[10]. The available immunosuppressive and immunostimulating agents have major limitations; including adverse drug reactions and toxicities, as well as increased risk of infection due to their prolonged use in case they cause immunosuppression. The drugs are also expensive and may not be easily accessible to all the people who may need them. As a result, many people worldwide have resorted to the use of medicinal plants that have either immunosuppressing or immunostimulating effects in the body in form of medicinal plant products (Nutriceuticals). The nutriceuticals are used to stimulate or suppress the cell-mediated and humoral immune body responses against invading foreign bodies. A number of medicinal plants have been reported globally and used by the traditional herbalists and the communities' in the modulation of the immune system in both developing and developed countries. The herbs have gained advantages over the conventionally used drug due to the presumed less side effects, lack of access to modern drugs, unaffordable cost and inaccessibility to the healthcare services especially to many people in rural resource limited areas[18,19]. Many primary and secondary plant metabolites have been found to modulate the immune system function through the cell mediated and humoral responses by either stimulating or suppressing the different stages of hemopoiesis[20,21]. Among the herbs used include the Meliaceae family that has been widely used in natural medicine for immunomodulatory activity, antiviral, anthelmintic, anti-inflammatory and anti-rheumatic activities[22,40,41,43,48]. The anti-inflammatory and anti-rheumatic properties of some members of this family, like *Azadirachta indica* and *Munronia pumila*, have been reported to have an effect on the immune system[22]. Also some of these medicinal plants used for immunomodulation have provided potential alternatives to conventional chemotherapies for a variety of diseases, especially those that lead to impaired immune response[23]. Among the medicinal herbs reported to modulate the immune response include African potato, Ovacado and many others that are used by the local communities and traditional herbalists as immune boosters[24,25]. *S. anguivi* is a common herb in Uganda that is used as a food and as herbal medicine in treatment of various disease conditions and as an immune booster by the local communities.

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S.anguivi is a shrub that grows well in tropical regions of the world[26-28]. It is used as a food and medicine by many people worldwide especially in the tropical regions of the world like Uganda[26,29]. The herb has many medicinal values including being reported to be used in treatment of hypertension, urinary tract infections, as an immune booster; diabetes mellitus by lowering blood glucose absorption from the gastrointestinal tract, enhancement of kidney function, lower blood pressure and it is also used by women after birth because they believe that it increases milk production[28]. The herb has been reported to contain a variety of compounds that have medicinal values including steroids and triterpenoids, alkaloids, reducing sugars, tannins, steroid saponins, isoanguivine, protodioscin, solasonine and solamargine[30-33]. Also steroidal glycosides indiosides A-E have been isolated. Antioxidant compounds such as ascorbic acid, carotenoids and polyphenols, polyphenols, several phenolic acids and flavonoids, caffeoylquinic acids, caffeic acid, flavonol glycosides and naringenin have also been reported[30,31,33]. Though it has been reported as an immune booster, its immunomodulatory activity has not been scientifically documented despite its wide use as a food and as a medicine to manage various disease conditions. The study investigated the immunomodulatory activity of *S. anguivi* on both the humoral and cell-mediated immune responses in Wister albino rats in which the complete blood count (CBC), immune blood cell count, haemagglutinating antibody (HA) titer, neutrophils adhesion and delayed-type hypersensitivity (DTH) response were determined.

MATERIALS AND METHODS

Study design

An experimental study investigated the immunomodulatory activity of *S. anguivi* on both the humoral and cell-mediated immune responses in Wister albino rats in which the complete blood count (CBC), immune blood cell count, haemagglutinating antibody (HA) titer, neutrophils adhesion and delayed-type hypersensitivity (DTH) reaction were determined using standard methods and procedures.

Processing and extraction

After the verification process, the collection of the selected plants was carried out according to the standard procedure[34]. Fresh mature leaves of *S.anguivi* were collected, identified by a botanist and voucher specimen was deposited at the Makerere University Herbarium. The leaves were cleaned with clean water. They were air-dried in a shade until constant weight was obtained. Dried leaves were then pulverized into coarse powder for extraction process. The medicinal plant was extracted serially using ether and ethanol solvents. About 500g of the leaf coarse powder were put in Erlenmeyer flasks and soaked in 1500mls of ether solvent for 72 hours with occasional shaking to facilitate the extraction process. The mixture was then filtered using Whatman No.1 filter paper using a Buchner funnel and a suction pump. The residue was air-dried for about 2 hours in preparation for ethanol extraction. It was soaked in 1500 ml of 96% ethanol and the procedure was repeated as for the ether extraction. The ether and ethanol solvents were recovered from the extracts using a Heidolph rotary evaporator (BÜCHI Rotavapor R-205 model) to obtain semi dry ether and ethanolic leaf extracts. They were then mixed in equal proportions to obtain a total crude extract that was used in the experimental studies. To attain complete dry total crude leaf extract of *S. anguivi*, the mixture was kept at room temperature for one week to allow complete evaporation of the ether and ethanol solvents that were used in the immunomodulatory studies.

Preparation of the total crude leaf extract stock solution and different concentrations

The total crude leaf extract stock solution was prepared by dissolving 2500mg of the extract with a few drops cooking oil and then topped up with normal saline to produce a concentration of 2500mg/5mL (500mg/mL). A concentration of 125, 250 and 500mg/Kg/mL of the total crude leaf extract were prepared by serial dilutions. Normal saline with cooking oil was used as control.

Study animals

About 24 healthy adult Wistar albino rats of either sex, weighing between 80-150g were used in the study. The animals were housed in standard environmental conditions (temperature 25°C; photoperiod of approximately 12 hours of natural light per day; relative humidity of 50-55%) in order to acclimatize them before the experiment according to standard conditions. The ani-

mals were treated in a humane way as per the standard European guidelines on use of Laboratory animals[35,36]. The sickly, pregnant and nursing mothers were excluded from the study.

Group treatment of experimental animals

The 24 experimental Wister albino rats were obtained from the Department of Pharmacology and Therapeutics, Makerere University College of Health Sciences. They were six weeks old, visually healthy looking albino rats of both sexes were randomly selected for use in evaluation of the immunomodulatory activity of the total crude extracts for the herbs. The animals were grouped into 4 groups; each group consisted of six animals (3 males and 3 females). Group I was dosed with 1mL of normal saline and cooking oil mixture (control group). Group II was dosed 125mg/Kg bwt/mL. Group III was dosed 250mg/Kg bwt/mL. Group IV was dosed 500mg/Kg bwt/mL. The animals were being feed on standard pellet food and were provided with water ad-libitum. The animals were dosed with respective doses daily for 14 days. On the 15th day, whole blood was collected by puncturing the retro orbital vein of the rats for the immunomodulatory experimental studies.

Preparation of sheep red blood cells (SRBC) as antigens

Fresh blood was collected from a sheep in a sterile bottle containing Alsver's solution (2 %dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% sodium chloride). The sheep red blood cells (SRBC) were thoroughly mixed and washed three times with normal saline and each time centrifuged at 3000 rpm for 5 minutes. The supernatant was then discarded. The SRBC got were washed again with sterilized phosphate buffer saline (pH 7.2). The total SRBC was counted using Neubauer chamber and finally 1x10^{8} SRBC (1.5 mL) were injected intraperitoneally for sensitization and challenging the rats[37].

Immunomodulatory bioassays

Determination of complete blood count (CBC)

The whole blood from groups I, II, III and IV were collected by performing a retro-orbital puncture. The blood was collected into a clean EDTA-containing vacutainers. The CBC was determined using automated hematological Coulter CBC-5 Hematology Analyzer equipment using standard procedures. The print outs were made and different parameters were entered in Excel spread sheet for each animal in preparation for statistical analysis. The RBC count, WBC count and differential count or cell-mediated immune response (immune blood cells - % neutrophils, % basophils, % eosinophils, % monocytes and % lymphocytes) were determined and evaluated.

Determination of humoral immune response - hemagglutination antibody (HA) titer

The study was conducted according to Puri et al. (1993) method[38]. Four groups of rats were used. Group I was given 1mL of normal saline orally for 14 days. Group II, III and IV were pre treated (orally) with 1mL of 125 mg/Kg, 250 mg/Kg and 500mg/Kg of the total crude extract daily for 14 days. The animals were then immunized by injecting 0.1ml of SRBCs suspension containing 20 µl of 5×10^{9} cells intraperitoneally on the 15th day. The day of immunization was day 0. The animals continued to receive normal saline + cooking oil and 125mg/Kg, 250 mg/Kg and 500mg/Kg bwt of the total crude leaf extracts in their respective groups for the next 14 days. Blood samples were then collected from each animal by retro-orbital puncture on the 15th day (after immunization) into a clean clot-activated vacutainer. The blood samples were centrifuged at 1500 rpm for 5minutes to obtain serum. The serum was collected and the haemagglutination titer was determined using microtiter plates. Two-fold dilutions (0.025 ml) of sera were made in the micro-titer plates using normal saline. To each well, 0.025 ml of 1% (v/v) SRBC was added. The plates were incubated for 1 hour at 37 °C and then observed for haemagglutination. The highest dilution giving haemagglutination was taken as the antibody titer, which was expressed in a graded manner, the minimum dilution (1/2) being ranked as 2.

Determination of neutrophil adhesion

The study was conducted according to Shuklaa et al. (2009) method[10]. The animals were divided into four groups each with six rats. Group I was given 1mL of normal saline + cooking oil orally. Groups II, III and IV were given 1mL of 125 mg/Kg, 250 mg/Kg, and 500mg/Kg bwt/day respectively orally daily for 14th days. On 15th day, blood samples were collected from each of the animals for all the groups by puncturing the retro-orbital vein into a clean EDTA containing

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vacutainers. The total leukocyte cell (TLC) and differential leukocyte cell (DLC) count were determined using automated hematological Coulter CBC-5 Hematology Analyzer equipment using standard procedures. After initial counts, blood samples were incubated with nylon fibers for 15minutes at 37 °C. The incubated blood samples were again analyzed after removing the nylon fibers for TLC and DLC respectively to give neutrophil index of blood samples. The percentage neutrophil adhesion was calculated using the following formula:

Neutrophil adhesion (%) = (NIu – Nlt) × 100/NIu

Where NIu is the neutrophil index of untreated blood samples and NIt is the neutrophil index of treated blood samples. The mean percentage (%) Neutrophil adhesion was calculated.

Determination of delayed-type hypersensitivity (DTH) response

The study was conducted using standard methods[39].Wister albino rats were divided into 4 groups each comprising of 6 rats. Group I was given 1mL normal saline orally. Group II, III and IV were dosed 1mL of 125 mg/Kg, 250 mg/Kg, and 500mg/Kg bwt/day respectively orally daily for 14 days[53]. The rats were then challenged on the 14th day with 20 µl of 5×10^9 SRBC/ml subcutaneously into the right hind foot pad. Footpad thickness was measured using a vernier caliper at 0 hour, 12 hours, 24 hours and 48 hours after the challenge. The differences obtained for pre- and post challenge footpad thickness was taken as the measurement of DTH and was expressed in mm.

Statistical data analysis

That data for RBC, WBC count, % neutrophils, % basophils, % eosinophils, % monocytes and % lymphocytes, HA titer, neutrophils adhesion and DTH were analyzed by the Excel statistical package using the student's t-test. The mean values were compared with the controls for all the 4 groups. Data was expressed as mean standard deviation(S.D.) of the means. The mean differences between the test group that received the total crude extracts and control were considered significant when $p < 0.05$.

Ethical considerations

All the necessary ethical issues and animal rights were considered throughout the experimental study. The experiments were conducted in accordance with the internationally accepted principles for laboratory animal use and care[35].

RESULTS

The immunomodulatory activity of *S. anguivi (indicum)* on the immune system both the cell-mediated and humoral immune responses in Wistar albino rats in which the RBC count, WBC count, differential count (% neutrophils, % eosinophils, % basophils, % monocytes and % lymphocytes), HA titer, neutrophil adhesion and delayed hypersensitivity test (DHT) were determined. The results showed that there was a dose-dependent reduction in the mean WBC count. The dose at 125mg/Kg bwt had a higher mean WBC count of $13.7\pm1.6x10^3/\mu L$ of blood as compared to the $13.1\pm0.2x10^3/\mu L$ of the control group. The mean WBC count at 500mg/Kg bwt was statistically different to that of the control group ($p\leq0.05$). The mean RBC count were higher than that of the control group for all the doses (125, 250 and 500mg/Kg bwt) but there was no statistical difference between the 2 groups ($p\geq0.05$) (TABLE 1). For the differential count, the mean % neutrophils were higher at a dose of 250mg/Kg bwt as compared to the control group but for all the doses, there was no statistical difference between the 2 groups ($p\geq0.05$). The mean % lymphocytes were higher for all the doses than the control group but with no statistical difference between the 2 groups ($p\geq0.05$) though however, there was a dose-dependent reduction in the mean % lymphocytes. The mean % monocytes, mean % eosinophils and mean % basophils were lower for all the doses as compared to the control group with no statistical difference between the 2 groups ($p\geq0.05$) (TABLE 1). The mean heamagglutination antibody (HA) titer levels for all the doses were higher than for the control group. However there was a statistical difference at a dose of 125mg/Kg bwt and 250mg/Kg bwt as compared to the control group ($p\leq0.05$)(TABLE 2). The mean % neutrophil adhesions were higher than the control group though there was no statistical difference between the 2 groups ($p\geq0.05$) (TABLE 3). The delayed type hypersensitivity test (DHT) response for all the doses were higher than for the control group for the 12, 24 and 48 hours after the injection of SRBC antigens in the footpad of the Wistar Albino rats. The highest responses in the footpad thickness for all the doses were

observed after 12 hours of injection of antigens in the animal footpads. The highest response (150% increment) in the increment of the footpad thickness was observed at a dose of 125mg/Kg bwt and 109.3% increment at a dose of 500mg/Kg bwt and there was statistical difference ($p \leq 0.05$) as compared to the control group. Overall there was a time-dependent reduction in the footpad thickness for all the doses from 12 hours to 48 hours after the injection of SRBC in the footpad of the animals (TABLE 4 and figure 1).

TABLE 1 : Effect of different doses of the total crude extracts of *S. anguivi* on the mean CBC

Medicinal herb	Dose (mg/kg)	Mean WBC $x10^3/\mu l \pm SD$	Mean RBC $x10^6/\mu l \pm SD$	Mean differential count ±SD (%)				
				NE	LY	MO	EO	BA
Solanum anguivi	125	13.7 ± 1.6^b	8.1 ± 0.4^b	13.2 ± 2.8^b	83.5 ± 4.8^b	2.7 ± 6.7^b	0.39 ± 0.35^b	0.56 ± 0.49^b
	250	11.9 ± 4.2^b	7.7 ± 1.2^b	16.2 ± 0.8^b	81.7 ± 1.0^b	1.1 ± 0.21^b	0.36 ± 0.64^b	0.67 ± 0.07^b
	500	9.0 ± 1.9^a	8.1 ± 0.1^b	15.2 ± 3.7^b	81.0 ± 4.6^b	1.6 ± 0.0^b	0.80 ± 0.21^b	1.45 ± 0.71^b
NS+C	1mL	13.1 ± 0.2	7.3 ± 0.3	15.5 ± 1.6	77.6 ± 0.1	4.6 ± 1.87	0.88 ± 0.07	1.55 ± 0.21

Key : a denotes $p<0.05$ and b denotes $p>0.05$; NE-Neutrophils, LY- Lymphocytes, MO- Monocytes, EO- Eosinophils, BA- Basophils WBC- White Blood Cell count, RBC- Red Blood Cell Count, SD- Standard Deviation; NS+C – Normal saline + cooking oil

TABLE 2 : Effect of varying doses of the total crude extract of *S. anguivi* on heamagglutination antibody titer

Medicinal herb	Dose (mg/kg/bwt)	Mean Heamagglutination antibody titer (±SD)	p value
S. anguivi	125	14.0 ± 5.7^a	0.037
	250	15.2 ± 11.3^a	0.044
	500	7.7 ± 8.5^b	0.11
NS+C (Control)	1mL	3.0 ± 1.4	

Key : ^{a} $p<0.05$ and ^{b} $p > 0.05$; NS+C – Normal saline + cooking oil

TABLE 3 : Effect of different doses of the total crude extracts of *S.anguivi* on the mean % Neutrophil adhesion

Medicinal herb	Dose (mg/kg) (treatment)	Mean % Neutrophil in untreated blood	Mean % neutrophil in blood treated with nylon fibers	% Neutrophil adhesion
S.anguivi	125	15.90±1.63	12.3±0.07	22.64^b
	250	18.38±1.34	12.58±0.07	31.56^b
	500	19.85±4.88	15.43±0.07	22.27^b
NS+C (Control)	1mL	13.08±1.56	10.58±1.41	19.11

Key : a denotes $p <0.05$ and b denotes $p >0.05$; NS+C – Normal saline + cooking oil

DISCUSSION

Substances that are capable of activating the hosts' defense mechanisms through the immune system have been used globally as a way to control diseases in both humans and animals. In this study, the immunomodulatory activity of *S. anguivi* on both the humoral and cell-mediated immune responses in Wister albino rats, in which RBC, WBC count, % neutrophils, % basophils, % eosinophils, % monocytes and % lymphocytes, HA titer, neutrophils adhesion and DTH were determined. The results showed that there was a dose-dependent reduction in the mean WBC count. The dose at 125mg/Kg bwt had a higher mean WBC count of $13.7 \pm 1.6 x 10^3/\mu L$ of blood as compared to the $13.1 \pm 0.2 x 10^3/\mu L$ of the control group. The mean WBC count at 500mg/Kg bwt was statistically different to that of the control group ($p \leq 0.05$). The mean RBC count were higher than that of the control group for all the doses (125, 250 and 500mg/Kg bwt) and there was no statistical difference between the 2 groups ($p \geq 0.05$) (TABLE 1). For the differential count, the mean % neutrophils were higher at a dose of 250mg/Kg bwt as compared to the control group but for all the doses, there was no statistical difference between the 2 groups ($p \geq 0.05$). The mean % lymphocytes were higher for all the doses than the control group but with no statistical difference between the 2 groups ($p \geq 0.05$) though however, there was a dose-dependent reduction in the mean % lymphocytes. The mean % monocytes, mean % eosinophils and mean % basophils were lower for all the doses as compared to the control group with no statistical difference between the 2 groups ($p \geq 0.05$) (TABLE 1). The increase in the mean RBC count, mean % lymphocytes at all the doses, mean % neutrophils at a dose of 250mg/Kg bwt and mean WBC count at a dose of 125mg/Kg bwt as well as the reduction in the mean %monocytes, mean % eosinophils and mean % basophils could be due to the presence of the various compounds present in the *S. anguivi* like steroids and

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TABLE 4: Effect of different doses of the total crude extracts of *S.anguivi* on the mean foot pad thickness (delayed hypersensitivity)

Medicinal herb	Dose (mg/kg)	Mean Foot pad thickness ±SD (mm) at given time interval (hours)							
		0 (hr)	%	12 (hr)	%↑	24 (hr)	%↑	48 (hr)	%↑
S.anguivi	125	0.06±0.053[b]	0	0.15±0.042[b]	150.0	0.13±0.028[a]	116.7	0.095±0.014[b]	58.3
	250	0.088±0.042[b]	0	0.159±0.032[b]	80.7	0.144±0.025[b]	63.6	0.11±0.01[b]	26.1
	500	0.075±0.014[b]	0	0.157±0.0[a]	109.3	0.133±0.014[b]	77.3	0.096±0.014[b]	28.0
NS+C	1mL	0.07±0.014	0	0.115±0.042	64.3	0.108±0.035	54.3	0.082±0.014	17.1

Key : a denotes p<0.05 and b denotes p>0.05; '!- increment, Sa – *Solanum anguivi*, NS+C – Normal saline + cooking oil, hr- hour

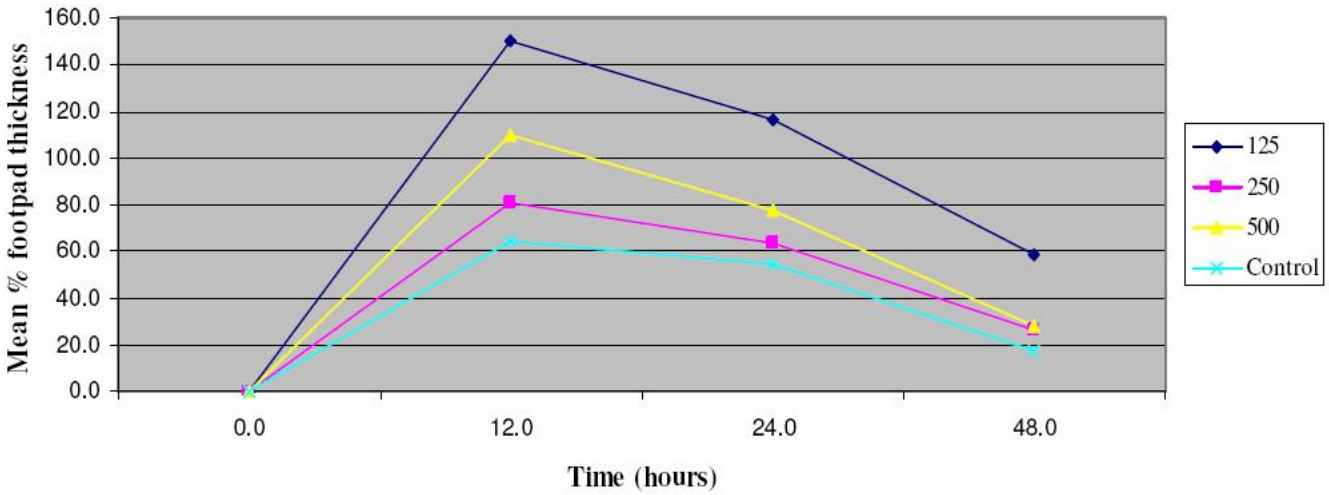

Figure 1 : Effect of different doses of the total crude extracts of *Solanum anguivi* (indicum) on the mean % increment of foot pad thickness at different time interval

triterpenoids, alkaloids, reducing sugars, tannins, steroid saponins, isoanguivine, protodioscin, solasonine and solamargine[30-33]. Also it contains antioxidant compounds like ascorbic acid, carotenoids and polyphenols, polyphenols, several phenolic acids and flavonoids, caffeoylquinic acids, caffeic acid, flavonol glycosides and naringenin have also been reported[30,31,33]. Some of these compounds could interfere with the different processes in the white blood cell formation stage of the hematopoietic system[16,31,45-47]. Nonetheless the dose dependent reduction in the mean WBC count could have been due to some of the compounds in the herb that caused the suppression of bone marrow. The data undoubtedly have demonstrated that the herb contain immunomodulatory compounds. The dose effect observed could be due to the increased activity of the extracts that may be attributed to by the increased polarity caused by the water molecule fraction hence increasing the solubility of the compounds in the extracts and the absorption of the extracts from the gastrointestinal tract in the dilute form. The various compounds in the herb could be acting as either stimulating or inhibiting natural factors that promote the proliferation or suppression of the various blood cell components such as granulocytes colony stimulating factors (G-CSF)[4,42,44]. The results also add to the other medicinal herbs such as African potato[24] and Avocado[25], that have been reported to have immunomodulatory activities and hence their increased use in stimulating the immune system in treatment of various body conditions worldwide and therefore the use of these herbs in nutriceuticals.

The mean % neutrophil adhesions were higher than the control group though there was no statistical difference between the 2 groups (p≥0.05) (TABLE 3). The study shows that the above compounds in *S. anguivi* can cause neutrophil migration towards the foreign bodies as observed with the nylon fibers. Arguably, this correlates with improved migration of phagocytes such as neutrophils in blood vessels thus facilitating migration to the site of inflammation as reported earlier[7,8]. Results also show that, the extract increased the neutrophil adhesiveness an indication of the boosting of neutrophils to migrate towards the foreign body[42]. The neutrophils represent a multifunctional cell type in in-

nate immunity that contributes to bacterial clearance by recognition, phagocytosis and killing of foreign bodies[7,8,49] where as the T and B-lymphocytes are involved and responsible for production of antibodies leading to enhancement of immunity[7,8,13,49,50]. Also neutrophil granules contain a variety of toxic substances that kill or inhibit growth of bacteria and also mediate other cells of the immune system such as the macrophages.

The mean heamagglutination antibody (HA) titer levels for all the doses were higher than for the control group. However there was a statistical difference at a dose of 125mg/Kg bwt and 250mg/Kg bwt as compared to the control group ($p \leq 0.05$)(TABLE 2). *S.anguivi* total crude extracts could have compounds with immunostimulatory effect on the B-cells of the immune system as observed by the increment in the antibody titers in the dosed Wistar albino rats unlike the control. Humoral immunity involves interaction of B cells with the antigen and their subsequent proliferation and differentiation into antibody secreting (plasma) cells. Antibodies function as the effectors of the humoral response by binding to antigen and neutralizing them or facilitating elimination by cross linking to form latex that are more readily ingested by phagocytic cells[1,21]. Therefore the plant extract may cause augmentation of the humoral immune response to SRBCs acting as antigens that cause enhanced responsiveness of T and B-lymphocyte subtypes of the immune system thus promoting the anti-body synthesis[17] in the Wistar albino rats and the results were similar to what has been observed in other studies.

The delayed type hypersensitivity test (DHT) response for all the doses were higher than for the control group for the 12, 24 and 48 hours after the injection of SRBC antigens in the footpad of the Wistar albino rats. The highest responses in the footpad thickness for all the doses were observed after 12 hours of injection of antigens in the animal footpads. The highest response (150% increment) in the increment of the footpad thickness was observed at a dose of 125mg/Kg bwt and 109.3% increment at a dose of 500mg/Kg bwt and there was statistical difference ($p \leq 0.05$) as compared to the control group. Overall there was a time-dependent reduction in the footpad thickness for all the doses from 12 hours to 48 hours after the injection of SRBC in the footpad of the animals (TABLE 4 and figure 1). The observed effect may be attributed to by some of the above compounds present in *S. anguivi* that led to the delayed type of hypersensitivity reaction towards the antigens that were injected in the rats' footpad. The delayed type hypersensitivity (DTH) response is a type IV hypersensitivity reaction, which is a direct correlate of cell-mediated immunity. Increase in the DHT indicates that *S. anguivi* total crude extracts have a stimulatory effect on lymphocytes and accessory cell types required for the expression of the reaction[17,51]. Thus this concurs with other research work done by other researchers using other types of medicinal plants[8,20,52,53]. Cell-mediated immunity involves effector mechanisms carried out by T lymphocytes and their products (lymphokines). DTH requires the specific recognition of a given antigen by activated T lymphocytes, which subsequently proliferate and release cytokines. These in turn increase vascular permeability, induce vasodilatation, microphage accumulation, and activation, promoting increased phagocytic activity and increased concentration of lytic enzymes for more effective killing. When activated Th_1 cells encounter certain antigens such as SRBCs they are converted to lymphoblasts[4] and secretes cytokines that induce a localized (defensive) inflammatory reaction called delayed type hypersensitivity[11,13]. The cytokines then attract the scavenger phagocytic cells to the site of reaction hence leading to the type IV delayed hypersensitivity reaction observed in the study when the cells encounter the antigens in form of plant extracts[14]. The observed effect in the delayed hypersensitivity in the rat foot pad could be due to the T-lymphocytes and monocytes and/or macrophages[13,14]. Also the cytotoxic T-cells may have caused direct damage to the foot pad where as T-helper (TH1) cells may also have increased damage by secreting cytokines that activate cytotoxic T cells that recruit and activate monocytes and macrophages causing a bulk of tissue damage observed in the study by the increased thickness of the footpad[13,14]. The study has provided evidence that S.*anguivi* has immunomodulatory compounds and hence its continued use by the local communities and traditional herbalist in management of variety of disease conditions.

CONCLUSION

The total crude leaf extract of *S. anguivi* has com-

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pounds with immunomodulatory activity. The compounds increased the red blood cells production due to the positive effect on erythropoiesis process. The extracts boosted the cell-mediated immune response of some of the 'phagocytes' though depressed others. The phagocytic potential may be enhanced by the increased neutrophil adhesiveness. The high heamagglutination titers observed was an indication that the plant contains compounds that may boost the humoral immunity. The results show that *S. anguivi* has compounds that affect both the cell-mediated and humoral immune responses and this provides evidence for its wide use as an immune booster in the management of number of disease by both the local communities and the traditional health practitioners.

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