

Full Length Research Paper

Effect of the total crude extracts of *Hibiscus sabdariffa* on the immune system in the Wistar albino rats

Aloysius M.B. Lubega¹, Godfrey S. Bbosa^{1*}, Nathan Musisi², Joseph Erume² and Jasper Ogwal-Okeng¹

¹Department of Pharmacology and Therapeutics, Makerere University College of Health Sciences, Kampala, Uganda.

²Department of Veterinary Microbiology and Parasitology, Makerere University College of Veterinary Medicine, Animal Resources and Bio-Security, Kampala, Uganda.

Accepted 30 May, 2013

Medicinal herbs are commonly used worldwide as immune boosters and immunomodulators in the management of various disease conditions. Many of these herbs commonly used have not been scientifically evaluated for their immune modulating activities. The study investigated the immunomodulatory activity of the total crude leaf extract of *Hibiscus sabdariffa* in Wistar albino rats. It was an experimental study that was conducted on four groups of animals each with 6 healthy adult rats. Group I was dosed each with 1mL of normal saline. Groups II, III and IV were dosed 1mL of 125, 250 and 500 mg/Kg bwt of total crude extract, daily for 14 days respectively. On the 15th day, whole blood was collected into a clean ethylenediaminetetracetic acid (EDTA)-vacutainer. The complete blood count (CBC), immune blood cell count, hemagglutination antibody (HA) titers, neutrophil adhesion and delayed-type hypersensitivity (DTH) response were determined. All the doses caused an increase in mean red blood cell (RBC) counts as compared to control group. Similarly, the mean percentage neutrophils, monocytes, basophils and eosinophils increased with dose while the opposite was true for percentage lymphocytes. The mean HA titers for the herb were higher than control though no statistical difference ($p \geq 0.05$) was observed. Similar effects were observed with neutrophil adhesions response as that of HA titers. For DTH, the highest footpad thickness (175.2% increment) was observed at a dose of 500 mg/Kg bwt after 12 h and was statistically significant ($p \leq 0.05$) as compared to control. *H. sabdariffa* contain compounds with immunomodulatory activity in Wistar albino rats.

Key words: Immunomodulation, *Hibiscus sabdariffa*, total crude extracts.

INTRODUCTION

Natural medicinal herbs have long been used as aliments in management of various disease conditions, as immune boosters and immunomodulators worldwide (Joy et al., 1998; Zhang, 1999; Okwari and Ofem, 2011). The medicinal herbs are used to modulate the immune system by either stimulating or suppressing the cell-mediated and humoral-mediated immune body responses against foreign bodies. A number of medicinal plants have been reported globally and are used by the traditional herbalists and the various communities' in the

modulation of the immune system in both developing and developed countries (Gokin et al., 2000). Modulation of the immune system responses to alleviate disease conditions has been of medical interest worldwide for many years (Maizels, 2009; Shuklaa et al., 2009). Currently there is increased scientific interest in agents that can modulate the immune system in severely immunocompromised individuals like in cases of human immunodeficiency virus (HIV) infected individual, stress, malnutrition and many others (Shuklaa et al., 2009). The

*Corresponding author. Email: godfossa@yahoo.com.

available immunosuppressive and immunostimulating agents have limitations; including adverse drug reactions and toxicities, as well as increased risk of infection due to their prolonged use in case they cause immunosuppression. As a result, many people worldwide have resorted to the use of medicinal plants as immunomodulators especially in developing countries.

Many different medicinal plants contain different compounds and have long been used to modulate the humoral and cell-mediated immune responses as observed in this study on Wistar albino rats (Barkatullah et al., 2013). Some have been used to facilitate phagocytic function of the immune cells of the reticulo-endothelial system as well as in controlling the hypersensitivity and inflammatory reactions. Some herbs are used as antioxidants that detoxify the generated free radicals and stress factors in the body (Steenkamp et al., 2013). The exaggerated body reactions lead to undesirable and damaging effects to the body. The body reactions have been classified according to Coombs and Gell (1975) into four different types: Type I, type II, type III and type IV (Garland-Science, 2008; Marc and Olson, 2009; Douglas, 2011) and usually they are mediated by immunoglobulin or antibodies such as IgM, IgG, IgA, IgE, IgD (Amersham-Biosciences, 2002; Trajkovski et al., 2004; Douglas, 2011).

The herbs have gained advantages over the conventionally used drugs 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 (Kamatenesi, 2002; Arokiyaraj et al., 2009). Many primary and secondary plant metabolites (Barkatullah et al., 2013; Selim et al., 2013), 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 (Statpute et al., 2009). Among the herbs used include the Meliaceae family such as *Azadirachta indica* and *Munronia pumila* that have been widely used in natural medicine for their immunomodulatory activity, antiviral, anthelmintic, anti-inflammatory and anti-rheumatic activities (Benencia et al., 1995). Among the medicinal herbs reported to boost the immune response include African potato, ovacado and many others (Zhang, 1999; Jatawa et al., 2011).

In Uganda, *Hibiscus sabdariffa* is one of the commonly used herb locally in central Uganda in anemic and sick individuals to improve their health and as an immune booster (Naluswa, 1993; Jatawa et al., 2011; Okwari and Ofem, 2011). Locally it is known as "Musaayi in Luganda". *H. sabdariffa* belongs to the family of *Malvaceae* (Mahadevan et al., 2009). The plant is often cultivated and dispersed in the out skirts of human dwelling sites. The herb has been reported to have a variety of compounds including alkaloids, saponins, cardenolides, deoxy sugar, tannins, cardiac glycosides, flavonoids, anthraquinones, phenolics, steroids, glyco-

sides and ascorbic acid (vitamin C) (Bako et al., 2009; Kuriyan et al., 2010; Mungole and Chaturvedi, 2011; Barkatullah et al., 2013; Selim et al., 2013). The herb has been reported to have several medicinal values including anti-oxidants activity, immune booster, antibacterial activities and many others (Mahadevan et al., 2009; Kuriyan et al., 2010). However, its effects on the immune system as an immunomodulator have not been scientifically evaluated. The study investigated the immunomodulatory activity of *H. sabdariffa* on both the humoral and cell-mediated immune responses in Wistar albino rats.

MATERIALS AND METHODS

Study design

An experimental study investigated the immunomodulatory activity of *H. sabdariffa* on the complete blood count (CBC), immune blood cell count (differential counts), haemagglutinating antibody (HA) titer, neutrophils adhesion and delayed-type hypersensitivity (DTH) reaction in Wistar albino rats.

Processing and extraction

After the verification process, the collection of the selected plants was carried out according to the standard procedure (Marjorie, 1999). Fresh mature leaves of *H. sabdariffa* were collected, identified by a botanist and voucher specimen was deposited at the Makerere University Herbarium. The leaves were cleaned with distilled water. They were air-dried in a shade until constant weight was obtained. Dried leaves were then pulverized into coarse powder to facilitate the extraction process. The medicinal plant was extracted serially using ether and ethanol solvents. About 500 g of the leaf coarse powder were put in Erlenmeyer flasks and soaked in 1500 mls of ether solvent for 72 h 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 h in preparation for ethanol extraction. It was then 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 *H. sabdariffa*, the mixture was kept at room temperature (25°C) for one week to allow complete evaporation of the ether and ethanol solvents, which was used in the immunomodulatory studies.

Preparation of the total crude leaf extract

The total crude leaf extract stock solution was prepared by dissolving 2500 mg of the extract with a few drops cooking oil and then topped up with normal saline to produce a concentration of 2500 mg/5mL (500mg/mL). A concentration of 125, 250 and 500 mg/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 80-150 g were used in the study. The animals were housed in standard environmental conditions (temperature 25°C; photoperiod approximately 12 h of natural light per day; relative humidity of 50-55%) in order to acclimatize them before the experiment according to standard conditions. The animals were treated in a humane way as per the standard European guidelines on use of Laboratory animals (EOCD, 2001; Khotimchenko et al., 2006). The sickly, pregnant and nursing mothers were excluded from the study.

Group treatment of experimental animals

The 24 experimental Wistar albino rats were obtained from the Department of Pharmacology and Therapeutics, Makerere University College of Health Sciences, Uganda. They were six weeks old, healthy and both sexes were used in the study. The animals were grouped into 4 groups; each group consisted of six animals (3 males and 3 females). Group I was dosed with 1 mL of normal saline and cooking oil mixture (control group). Group II was dosed 125 mg/Kg bwt/mL. Group III was dosed 250 mg/Kg bwt/mL. Group IV was dosed 500 mg/Kg bwt/mL. The animals were being fed on standard pellet food and were provided 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 Alver'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 min. 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 1×10^8 SRBC (1.5 mL) were injected intraperitoneally for sensitization and challenging the rats (Aher and Wahi, 2010).

Immunomodulatory bioassays

Determination of complete blood count (CBC)-cell-mediated immune responses

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 ethylenediaminetetraacetic acid (EDTA)-containing vacutainers. The CBC was determined using automated hematological Coulter CBC-5 Hematology Analyzer equipment using standard procedures. The red blood cell (RBC) count, white blood cell (WBC) count and differential count (immune blood cells: percentage neutrophils, basophils, eosinophils, monocytes and lymphocytes) were determined.

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

The study was conducted according to Puri et al. (1993) method. Four groups of rats were used as per the treatment groups above. On the 14th day, the animals were immunized by injecting 0.1 ml of SRBCs suspension containing 20 μ l of 5×10^9 cells intraperitoneally on the 15th day. The day of immunization was day 0. The animals continued to receive normal saline plus cooking oil and 125, 250

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 5 min to obtain serum. The serum was collected and the hemagglutination 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 h 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. Four groups of rats were used as per the treatment groups above. On the 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 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 15 min 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:

$$\text{Neutrophil adhesion (\%)} = (\text{Nlu} - \text{Nlt}) \times 100/\text{Nlu}$$

Where, Nlu is the neutrophil index of untreated blood samples and Nlt is the neutrophil index of treated blood samples. The mean percentage (%) neutrophil adhesion was calculated.

Determination delayed-type hypersensitivity (DTH) response

The study was conducted using standard methods (Ross et al., 2009). Four groups of rats were used as per the treatment groups above (Mayank et al., 2006). The rats were challenged on the 14th day with 20 μ l of 5×10^9 SRBC/mL subcutaneously into the right hind foot pad. Footpad thickness was measured using a vernier caliper at 0, 12, 24 and 48 h 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, percentage 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 for each dose of the extract were compared with the control. 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

Table 1. Effect of different doses of the total crude extracts of *H. sabdariffa* on RBC count, WBC count and differential count.

Medicinal herb	Dose (mg/kg)	Mean WBC $\times 10^3/\mu\text{l} \pm \text{SD}$	Mean RBC $\times 10^6/\mu\text{l} \pm \text{SD}$	Mean differential counts $\pm \text{SD}$ (%)				
				NE	LY	MO	EO	BA
<i>Hibiscus sabdariffa</i>	125	13.6 \pm 1.6 ^b	8.2 \pm 0.4 ^b	14.3 \pm 2.8 ^b	81.8 \pm 4.8 ^b	1.1 \pm 0.4 ^b	0.4 \pm 0.4 ^b	0.6 \pm 0.5 ^b
	250	11.7 \pm 4.2 ^b	8.0 \pm 1.2 ^b	18.3 \pm 5.9 ^b	80.0 \pm 1.0 ^b	1.2 \pm 0.2 ^b	0.2 \pm 0.1 ^b	0.4 \pm 0.1 ^b
	500	7.8 \pm 1.9 ^b	7.9 \pm 0.1 ^b	17.1 \pm 3.7 ^b	78.6 \pm 4.6 ^b	1.8 \pm 0.0 ^a	0.9 \pm 0.2 ^b	1.7 \pm 0.7 ^b
NS+C oil (control)	1 mL	13.1 \pm 0.1	7.3 \pm 0.3	15.5 \pm 1.6	77.6 \pm 3.7	4.6 \pm 1.8	0.8 \pm 0.1	1.6 \pm 0.2

^a, $p < 0.05$; ^b, $p > 0.05$; NS+C, normal saline + cooking oil; NE, neutrophils; LY, lymphocytes; MO, monocytes; EO, eosinophils; BA, basophils; WBC, white blood cell count; RBC, red blood cell count, SD, standard deviation.

principles for laboratory animal use and care (EOCD 2001).

RESULTS

The immunomodulatory activity of *H. sabdariffa* on both the humoral and cell-mediated immune responses in Wistar albino rats in which RBC, WBC count, percentage neutrophils, basophils, eosinophils, monocytes and lymphocytes, HA titer, neutrophils adhesion and DTH were determined. The mean WBC counts in rats dosed with 125 mg/Kg were slightly higher than the control group while the 250 and 500 mg/kg were generally slightly lower as compared to the control group. Though the differences were not statistically significant ($p > 0.05$). Dosing rats with 125, 250 and 500 mg/Kg of the total crude leaf extracts of *H. sabdariffa* caused a slight elevation in RBC counts as compared to the control but the difference was not statistically significant ($p > 0.05$). Regarding the differential counts, dosing rats with 125, 250, and 500 mg/Kg had no significant effect on the percentage neutrophils, lymphocytes, monocytes, eosinophils and basophils counts ($p > 0.05$). The mean percentage monocyte at 500 mg/Kg bwt was statistically significant ($p \leq 0.05$) as compared to the control

group. Though the differential counts appeared to rise with increasing dose from 125 to 500 mg/Kg bwt as compared to the control group (Table 1). The mean HA titer of the rats dosed 125, 250 and 500 mg/Kg bwt of the total crude leaf extract of *H. sabdariffa* were higher than that of the control group and the difference was statistically significantly ($p < 0.05$) (Table 2). The mean percentage neutrophil adhesion for the rats dosed with 125, 250 and 500 mg/Kg bwt were all higher than the control group though they were not statistically significant ($p > 0.05$). The highest mean percentage neutrophil adhesion was observed at 31.09% at a dose of 125 mg/Kg bwt (Table 3). The delayed hypersensitivity reaction test (DHT) showed that the mean percentage footpad thickness was higher than the control group at a dose of 125, 250 and 500mg/kg bwt. The highest observed effect occurred after 12 h of dosing (165.2% mean increment in footpad thickness) at 500 mg/Kg bwt dose giving the highest footpad thickness. The percentage mean footpad thickness was statistically significant ($p \leq 0.05$) at a dose of 500 mg/Kg bwt as compared to the control group after the 12 and 24 h of dosing. Though generally, there was a slight increase in the mean footpad thickness for all the doses as compared to the control group (Table 4 and Figure 1).

DISCUSSION

The modulation of body immune responses through suppression or stimulation is capable of maintaining a disease free state of an individual organism. Substances which 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. Generally, all the different types of blood cell count increased for all the doses of the total crude extracts of *H. sabdariffa* that were given to the animals as compared to the control group except the WBC count at 250 and 500 mg/Kg bwt, percentage neutrophils at 125 mg/Kg bwt, percentage monocytes, percentage eosinophils and percentage basophils at 125 and 250 mg/Kg bwt that were lower than the control group. However, the mean RBC count generally increased and were higher at a low dose of 125 mg/Kg bwt as compared to a high dose of 500 mg/Kg bwt of the total crude leaf extracts. The increment in the blood cells could be due to the stimulation of the bone marrow and lymphoid organs by the compounds such as alkaloids, saponins, cardenolides, deoxy sugar, tannins, cardiac glycosides, flavonoids, anthraquinones, phenolics, steroids, glycosides, ascorbic acid and other vitamins that are found in the herb (Essa et

Table 2. Effect of different doses of the total crude extract of *H. sabdariffa* on heamagglutination antibody titer.

Medicinal herb	Dose (mg/kg/bwt)	Mean heamagglutination antibody titer (\pm SD)	p value
<i>Hibiscus sabdariffa</i>	125	17.6 \pm 11.3 ^a	0.017
	250	36.8 \pm 33.9 ^a	0.021
	500	88.0 \pm 79.2 ^a	0.007
NS+C	1 mL	3.0 \pm 1.4	

^a, p<0.05; ^b, p>0.05; NS+C, normal saline + cooking oil (control).

Table 3. Effect of different doses of the total crude extracts of *H. sabdariffa* on the mean % neutrophil adhesion.

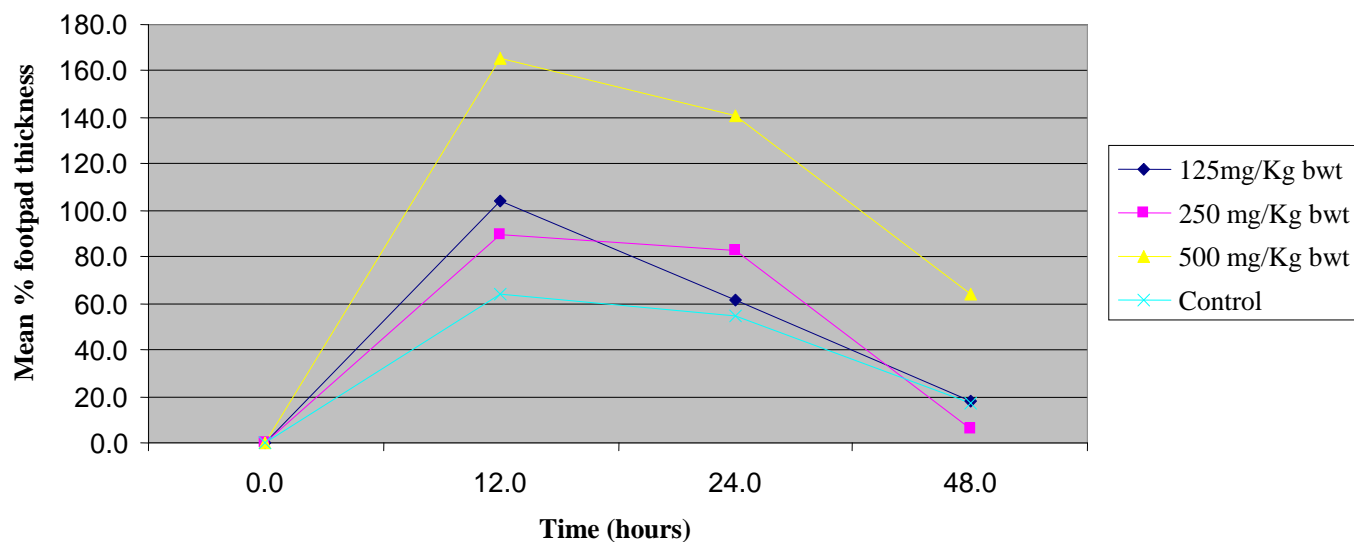
Medicinal herb	Dose (mg/kg) (treatment)	Mean % neutrophil in untreated blood	Mean % neutrophil in blood treated with nylon fibers	% Neutrophil adhesion
<i>Hibiscus sabdariffa</i>	125	14.28 \pm 2.76	9.84 \pm 1.41	31.09 ^b
	250	18.25 \pm 0.78	14.38 \pm 1.63	21.21 ^b
	500	17.07 \pm 3.68	12.83 \pm 0.283	24.84 ^b
NS+C	1 mL	13.08 \pm 1.56	10.58 \pm 1.41	19.11

^a, p<0.05; ^b, p>0.05; NS+C, normal saline + cooking oil (control).

Table 4. Effect of different doses of the total crude extracts of *H. sabdariffa* on the mean foot pad thickness (delayed hypersensitivity).

Medicinal herb	Dose (mg/kg)	Mean foot pad thickness \pm SD (mm) at given time interval (h)							
		0 (h)	%	12 (h)	% \uparrow	24 (h)	% \uparrow	48 (h)	% \uparrow
<i>Hibiscus sabdariffa</i>	125	0.07 \pm 0.02 ^b	0	0.14 \pm 0.00 ^b	140.4	0.11 \pm 0.00 ^b	61.8	0.08 \pm 0.01 ^b	17.7
	250	0.07 \pm 0.02 ^b	0	0.13 \pm 0.02 ^b	90.0	0.13 \pm 0.03 ^b	82.9	0.07 \pm 0.02 ^b	5.7
	500	0.08 \pm 0.04 ^b	0	0.18 \pm 0.12 ^a	165.2	0.16 \pm 0.01 ^a	140.9	0.11 \pm 0.01 ^b	63.6
NS+C	1 mL	0.07 \pm 0.01	0	0.12 \pm 0.04	64.3	0.11 \pm 0.04	54.3	0.08 \pm 0.01	17.1

^a, p<0.05; ^b, p>0.05; SD, standard deviation; \uparrow , increment; NS+C, normal saline + cooking oil.

**Figure 1.** Effect of different doses of the total crude extracts of *H. sabdariffa* on the mean % increment of foot pad thickness at time interval.

al., 2006; Bako et al., 2009; Kuriyan et al., 2010; Mungole and Chaturvedi, 2011). These compounds may stimulate the hemopoietic process leading to the increased activity of the different cell lines and hence the observed increment in the various blood cell types in the Wistar albino rats treated with the extract as compared to the control rats. However, the same compounds in the herb, some may depress the bone marrow and the lymphoid organs that produce the white blood cells hence leading to the observed reduction in the differential counts (Brunton et al., 2006; Prasad et al., 2006). The results also provide scientific evidence as to why the herb is widely used in central Uganda by both the local communities and the traditional herbalists in the management of anemia and other disease conditions and hence the local name "Musaayi" meaning blood in central Uganda (Brunton et al., 2006; Kuriyan et al., 2010). However, the dose dependent reduction in the total WBC count as compared to the control group could be due to the fact that *H. sabdariffa* contains compounds such as flavanoids, tannins, pectins hydroquinone, ascorbic acid, carotenoids and polyphenols, polyphenols, several phenolic acids and caffeoylquinic acids, caffeic acid, flavonol glycosides and others that could interfere with the different processes in the white blood cell formation stages of the hematopoietic system (Essa et al., 2006; Mahadevan et al., 2009; Mungole and Chaturvedi, 2011). Also the observed increased effect of the total crude extracts at low dose (125 mg/Kg bwt) as compared with the high dose (500 mg/Kg bwt) 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) (Steven, 2003; Prasad et al., 2006; Okwari and Ofem 2011).

The mean HA titer of the rats dosed 125, 250 and 500 mg/Kg bwt of the total crude leaf extract of *H. sabdariffa* were higher than that of the control group and dose dependent. The compounds in the herb may interact with the B cells acting as antigen and hence activate the subsequent proliferation and differentiation into antibody secreting (plasma) cells. The antibody molecules are products of B-lymphocytes that form the plasma cells leading to the formation of antibodies such as immunoglobulin(Ig) including the IgG, IgA, IgM, IgE and IgD (Steven, 2003), which are central in humoral immune responses. Therefore the herb may cause augmentation of the humoral immune response to sheep red blood cells SRBCs acting as antigens that caused enhanced responsiveness of T and B-lymphocyte subtypes of the immune system thus promoting the anti-body synthesis

(Sharififar et al., 2009) in the Wistar albino rats similar to what has been observed in other studies. The increased adhesion of the neutrophils to nylon fibers could be due to the compounds in the herb that may improve the migration of phagocytes such as neutrophils to the foreign body in blood vessels (Guyton and Hall, 2006; Srikumar et al., 2007). The neutrophils represent a multifunctional cell type in innate immunity that contributes to bacterial clearance by recognition, phagocytosis and killing of foreign bodies (Guyton and Hall, 2006; Srikumar et al., 2007), whereas the T and B-lymphocytes are involved and responsible for production of antibodies leading to enhancement of immunity (Guyton and Hall, 2006; Srikumar et al., 2007; Garland-Science, 2008; Soehnlein et al., 2008). The observed increase in the footpad thickness of the Wistar albino rats which is a measure of the DTH as a result of the challenge of the SRBC antigens could be due to some of the compounds found in the herbal extract. The compound might have stimulated the lymphocytes that mediates the delayed type of hypersensitivity or type IV reaction according to the Coomb and Gell (1975) classification (Garland-Science, 2008). The DTH response is a type IV hypersensitivity reaction, which is a direct correlation of cell-mediated immunity. Increase in the DHT indicates that *H. sabdariffa* total crude extracts have a stimulatory effect on lymphocytes and accessory cell types required for the immune reaction (cell-mediated immune response) (Makare et al., 2001; Sharififar et al., 2009). 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 thus contributing to the observed effect of the cardinal signs of inflammation. When activated, Th₁ cells encounter certain antigens such as SRBCs and they are converted to lymphoblasts (Steven, 2003) and secrete cytokines that induce a localized (defensive) inflammatory reaction called delayed type hypersensitivity (Coombs and Gell, 1975; Garland-Science 2008). 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 (Marc and Olson, 2009). The observed effect in the delayed hypersensitivity in the rat foot pad could be due to the T-lymphocytes and monocytes and/or macrophages (Garland-Science, 2008; Marc and Olson, 2009). Also the cytotoxic T-cells may have caused direct damage to the foot pad whereas 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 (Garland-Science, 2008; Marc and Olson, 2009). The study has provided evidence for the immunomodulatory activity of *H. sabdariffa* medicinal herb and its continued use by the local communities and traditional herbalist in management of variety of disease conditions.

Conclusion

The total crude leaf extract of *H. sabdariffa* has compounds with immunomodulatory activity on both cell-mediated and humoral-mediated immune responses. It increased red blood cell production and boosted some of the phagocytes. It increased the hemagglutination titers, an indication of boosting the humoral immunity. The results provides evidence for the wide use *H. sabdariffa* as an immune booster in the management of number of disease conditions by both local communities and the traditional health practitioners.

ACKNOWLEDGEMENT

I acknowledge the contribution of SIDA/SAREC for funding the programme and the study.

ABBREVIATIONS

HIV, Human immunodeficiency virus; **CBC**, complete blood count; **HA**, haemagglutinating antibody; **DTH**, delayed-type hypersensitivity; **SRBC**, sheep **RBC**, red blood cells; **EDTA**, ethylenediaminetetracetic acid; **WBC**, white blood cell; **TLC**, total leukocyte cell; **DLC**, differential leukocyte cell; **G-CSF**, granulocytes colony stimulating factors.

REFERENCES

- Aher VD, Wahi A (2010). Pharmacological study of *Tinospora cordifolia* as an immunomodulator. *Int. J. Cur. Pharm. Res.* 2 (4):52-54.
- Amersham-Biosciences (2002). Immunoglobulins-Antibody Purification Handbook: Handbooks from Amersham Biosciences. Amersham and Amersham Biosciences are trademarks of Amersham plc. http://kirschner.med.harvard.edu/files/protocols/GE_antibodypurification.pdf: 27-45.
- Arokiyaraj S, Agastian P, Perinbam K, Balaraju K (2009). Immunosuppressive effect of medicinal plants of Kolli hills on mitogen-stimulated proliferation of the human peripheral blood mononuclear cells *in vitro*. PG and Research. Sathyabama University, Chennai-600-119; Department of plant Biology and Biotechnology, School of life science, Loyola collage Chennai- 600-034, India.
- Bako IG, Mabrouk MA, Abubakar A (2009). Antioxidant Effect of Ethanolic Seed Extract of *Hibiscus sabdariffa linn* (Malvaceae) Alleviate the Toxicity Induced by Chronic Administration of Sodium Nitrate on Some Hematological Parameters in Wistar Rats. *Adv. J. Food Sci. Technol.* 1(1): 39-42.
- Barkatullah BB, Ibrar M, Niaz A, Naveed M, Rehmanullah (2013). Antispasmodic potential of leaves, barks and fruits of *Zanthoxylum armatum* DC. *Afr. J. Pharm. Pharmacol.* 7(13):685-693. DOI10.5897/AJPP12.725.
- Benencia F, Courrges MC, Nores MM, Coulombié FC (1995). Immunomodulatory activities of *Cedrela tubiflora* leaf aqueous extracts. *J. Ethnopharmacol.* 49:133-139.
- Brunton LL, Lazo JS, Parker KL (2006). Drugs acting o the blood and the blood forming organs and immunomodulators Goodman & Gilman, The Pharmacological Basis of Therapeutics. 11ed. Section 10 & 11:1405, 1433.
- Coombs RRA, Gell PGH (1975). Classification of Allergic Reactions Responsible for Clinical Hypersensitivity and Disease. In *Clinical Aspects of Immunology* (Gell PGH, Coombs RRA, Lachman PJ, Eds.). Lippincott, Philadelphia. 761-778.
- Douglas FF (2011). Immunoglobulins. <http://www.cehs.siu.edu/fix/medmicro/igs.htm> Retrieved 10th May 2011.
- EOCD (2001). Guideline on the use of laboratory animals in biomedical research. EOCD Geneva, Switzerland.
- Essa MM, Subramanian P, Manivasagam T, Dakshayani KB, Sivaperumal R, Subash S (2006). Protective influence of *Hibiscus sabdariffa*, an edible medicinal plant, on tissue lipid peroxidation and antioxidant status in hyperammonemic rats. *Afr. J. Tradit. Compl. Altern. Med.* 3(3):10-21.
- Garland-Science (2008). Allergy and Hypersensitivity. Garland Science. http://beta.garlandscience.com/res/pdf/9780815341239_ch13.pdf 13: 555-595.
- Gokin RH, Lahiri SK, Santani DD, Shah MB (2000). Evaluation of Immunomodulatory activity of *Clerodendrum phtomidis* and *Premna intergrifolia* root. *Int. J. Pharmacol.* 3(4):352-356.
- Guyton AC, Hall JE (2006). Blood cells, Immunity and Blood clotting factors Text book of Medical Physiology. 11th Ed. Elsevier Saunders Publishers Unit VI 417-450.
- Jatawa AS, Paul R, Tiwari A (2011). Indian Medicinal Plants: A rich source of Natural Immunomodulator. *Int. J. Pharmacol.* 7(2):198-205.
- Joy PP, Thomas J, Mathew S, Skaria BP (1998). Medicinal Plants. Kerala Agricultural University. Aromatic and Medicinal Plants Research Station. Kerala, India.
- Kamatenesi MM (2002). The socio-cultural aspects in utilization of medical plants in reproductive health care in western Uganda. Paper presentation. Department of Botany, Makerere University Kampala, Uganda.
- Khotimchenko MY, Zueva EP, Lopatina KA, Khotimchenko YS, Shilova NV (2006). Gastroprotective effect of pectin preparation against indomethacin-induced lesions in Rats. *Int. J. Pharmacol.* 2(4):471-476.
- Kuriyan R, Rkumar D, Rajendran R, Kurpal AV (2010). An evaluation of *Hibiscus sabdariffa* leaves in hyperlipidemic Indians. *BMC Complementary and Alternative Medicine* 10:27(doi:10.1186 11472-6882-10-27).
- Mahadevan N, Shivali, Kamboj P (2009). *Hibiscus sabdariffa Linn*: An overview. *Nat. Prod. Radiance* 8(1):77-83.
- Maizels RM (2009). Parasite immunomodulation and polymorphisms of the immune system. *J. Biol.* 8(62): doi:10.1186/jbiol166.
- Makare N, Boodhankars S, Rangari V (2001). Immunomodulatory activity of alcoholic extract of *Mangifera indicum* in mice. *J. Ethnopharmacol.* 78: 133.
- Marc D, Olson K (2009). Hypersensitivity: Reactions and Methods of Detection. Neuroscience, Inc. December, 2009. https://www.neurorelief.com/uploads/content_files/Hypersensitivity.pdf.
- Marjorie MC (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews.* Am. Soc. Microbiol. 12:564-582.
- Mayank T, Bhargava S, Dixit VK (2006). Immunomodulatory Activity of *Chlorophytum borivilianum*, Santapau & Fernandes Department of Pharmaceutical Sciences, Dr H.S. Gour Vishwavidyalaya, Sagar, Madhya Pradesh, India.
- Mungole A, Chaturvedi A (2011). *Hibiscus sabdariffa L*, a rich source of secondary metabolites. *Int. J. Pharm. Sci. Rev. Res.* 6(1):83-87.
- Naluswa J (1993). A report on a pilot country study of non-wood forest products (NWFP) in Uganda. FAO Corporate Document Repository.
- Okwari OO, Ofem OE (2011). Aqueous leaf extracts of *Dombeya buttneri* improves some blood parameters in rats. *Int. J. Cur. Res.*

- 2(2):086-091.
- Prasad V, Jain V, Dorle AK (2006). Evaluation of *Momordica charantia ghrita* for Immunomodulatory. J. Plant Sci. 1(1):80-85.
- Puri A, Saxena R, Saxena RP, Saxena KC, Srivastava V, Tandon JS (1993). Immunostimulant agent's from *Andrographis peniculata*. J. Nat. Prod. 56:995-999.
- Ross GR, Selvasubramanian S, Jayasundar S (2009). Immunomodulatory activity of *Punica granatum* in rabbits—apreliminary study. J. Ethnopharmacol. 78(2001):85-87.
- Selim SA, Aziz MHA, Mashait MS, Warrad MF (2013). Antibacterial activities, chemical constitutes and acute toxicity of Egyptian *Origanum majorana L.*, *Peganum harmala L.* and *Salvia officinalis L.* essential oils. Afr. J. Pharm. Pharmacol. 7(13):725-735.
- Sharififar F, Pournourmohammadi S, Arabnejad M (2009). Immunomodulatory activity of aqueous extract of *Achillea wilhelmasii C Koch* in mice. Indian J. Experimental Biology 47:668-671.
- Shuklaa S, Mehtaa A, John J, Mehta P, Vyas SP, Shukla S (2009). Immunomodulatory activities of the ethanolic extract of *Caesalpinia bonducella* seeds. J. Ethnopharmacol. 125:252-256.
- Soehnlein O, Kenne E, Rotzius P, Eriksson EE, Lindbom L (2008). Neutrophil secretion products regulate anti—bacterial activity in monocytes and macrophages. Clin. Experimental Immunol. 151:139-145.
- Srikumar R, Parthasarathy NNJ, Manikandary S, Mulhnel A, Rajamani R, Sheeladevi R (2007). Immunomodulatory effect of *Triphala* during experimentally induced noise stress in albino Rats. J. Health Sci. 53(i):142-145.
- Statpute KL, Jadhav MM, Korodi RS, Katare YS, Patil MJ, Rukhsana R, Bafna AR (2009). Immodulatory activity of fruits of *Randia dumetorum Lamk.* J. Pharmacogonosy Phytother. 1(3):036-040.
- Steenkamp V, Nkwane O, van Tonder J, Dinsmore A, Gulumian M (2013). Evaluation of the phenolic and flavonoid contents and radical scavenging activity of three southern African medicinal plants. Afr. J. Pharm. Pharmacol. 7(13): 703-709. DOI10.5897/AJPP12.1207.
- Steven CD (2003). "Cytokines. Cincial Immunology and Serology : A laboratory perspective 6(2): 86-87.
- Trajkovski V, Ajdinski L, Spiroski M (2004). Plasma Concentration of Immunoglobulin Classes and Subclasses in Children with Autism in the Republic of Macedonia: Retrospective Study. Croatian Med. J. Clin. Sci. 45(6):746-749.
- Zhang X (1999). Role of the WHO monographs on selected medicinal plants. Traditional Medicine, Department of Essential Drugs and Medicines Policy, World Health Organization. <http://apps.who.int/medicinedocs/pdf/s4927e/s4927e.pdf> (accessed on 1st May 2011). 2:87-157.