

Immunomodulatory and erythropoietic effects of aqueous extract of the fruits of *Solanum torvum* Swartz (Solanaceae)

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ABSTRACT

Aim: The effect of *Solanum torvum* (Fam: Solanaceae) on delayed type hypersensitivity (DTH) response, hemagglutinating antibody (HA) titer, white blood cells (WBC), red blood cells (RBC) and hemoglobin concentration was investigated in Sprague-Dawley rats to establish immunomodulatory and erythropoietic activity. **Materials and Methods:** Sheep red blood cells (SRBC)-immunized and challenged rats were treated with *Solanum torvum* extract, levamisole and dexamethasone. Phenylhydrazine (PHZ)-induced anemia in rats was treated with the extract. **Results:** The aqueous *Solanum torvum* extract and levamisole significantly enhanced DTH response, increased HA titer and WBC count, while dexamethasone significantly decreased DTH response, did not increase HA titer, and did not enhance WBC profile. The extract and Feroglobin, the reference heamatinic, were able to reverse PHZ-induced anemia, and increase the RBCs and Hb concentration above baseline values within 24 days. **Conclusion:** *Solanum torvum* extract showed a concentration-dependent immunostimulant and erythropoietic activity.

Key words: Delayed type hypersensitivity response, hemagglutinating antibody titer, phenylhydrazine, sheep red blood cells, *Solanum torvum*

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INTRODUCTION

Solanum torvum Swartz (family: Solanaceae) commonly known as turkey berry has been called by several local names such as: *Susraba* (Ewe), *Kvabu Nsusuwa* (Kwahu), *Yaa Asantewa* (Asante Twi), and *Seseloatso* (Ga-Adamgbe). This plant is found in tropical Africa, Asia and South America. The fruits have been found to contain phytoconstituents such as steroid glycosides and saponins, fixed oil; vitamin B group; vitamin C; iron salts: saponins and steroidal alkaloids.^[1] In Ghana, various parts of the plant have been used either as a haemostatic after childbirth or as a source of saponin for the hemi synthesis of cortisone and sex hormones or for compounding sedatives, diuretics or digestive tonics.^[1] Its leaves have been used in the treatment of abdominal pain, whitlow and whooping cough; its fruits

are used in the treatment of anemia, inducing lactation, and treatment of wounds and snakebites.^[1] In most traditional Ghanaian homes, it has become customary to give to mothers, after childbirth, diets containing *Solanum torvum* fruits with the intention of enhancing vitality and reversing conditions of anemia. Though undocumented, it is generally observed that mothers who eat these fruits show enhanced health status. To date there is little scientific evidence to support the traditional use of *S. torvum* in the management of anemia and immunodeficiency and the possible mechanisms involved.

The study of agents that modulate the immune system to alleviate certain diseases of immunodeficiency has gained interest. A number of plant materials traditionally administered to mothers after childbirth to overcome the weakness and stress of pregnancy and childbirth, such as dry fruit like almond (*Prunus amygdalus*) and date palm (*Phoenix dactylifera*), seeds of *Buchanania lanzan* and *Euryale ferox* and dried rhizome of *Zingiber officinale*, have been shown to possess immunostimulatory properties,

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thus supporting their traditional uses.^[2] The current study presents an investigation of the immunomodulatory and erythropoietic activities of the fresh fruits of *Solanum torvum*.

MATERIALS AND METHODS

Fruit sample collection

Fresh fruits of *Solanum torvum* (Fam. Solanaceae), obtained from the local market at Ayigya, Kumasi, were authenticated at the Department of Pharmacognosy, Faculty of Pharmacy, Kwame Nkrumah University of Science and Technology – Ghana. A voucher specimen with number KNUST/HM1/L035 was deposited at the Faculty of Pharmacy's Herbarium, KNUST, Kumasi, Ghana.

Preparation of extract

Six hundred g of fresh *S. torvum* fruits and 600 ml of distilled water was blended and the homogenous mixture obtained filtered. The filtrate (700 ml) was then evaporated to dryness on a water bath. The dried extract obtained (10 g) was stored and labeled as STE or extract; 1.667% yield was obtained.

Animals

Sprague–Dawley rats of either sex (200–215 g) obtained from the animal house of the Department of Pharmacology, KNUST were used. The animals were housed in well-ventilated cages under normal temperature, humidity and light, and fed on normal rat chow (obtained from the animal house) and water *ad libitum*. All procedures and techniques used in these studies were in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NIH, Department of Health Services publication No. 83-23, revised 1985). The protocols for the study were approved by the Departmental Ethics Committee.

Drugs

Levamisole 40 mg/ml (Medicore Laboratories Pvt. Ltd, India) was used as the reference immunostimulant whereas dexamethasone sodium phosphate 4 mg/ml (Pharma Inter, Brussels, Belgium) was used as the reference immunosuppressant. Ferroglobin® (Vitabiotics Ltd, Great Britain), a liquid tonic containing iron 0.2%, zinc 0.06%, copper 0.02%, manganese 0.025% and vitamin B-Complex 0.39% in a blend of honey and malt, was used as the reference hematinic. Phenylhydrazine (BDH Poole, England) was used to induce anemia.

Sheep blood (Antigen)

For immunization and challenge of the experimental animals, fresh sheep red blood cells (SRBC) obtained from

the Kumasi Abattoir Company Ltd, Kumasi were used. Prior to their use, the SRBCs were washed three times in large volumes of 0.9% normal saline, and centrifuged at a speed of 5000 rpm for 10 min. The plasma was decanted and the packed cells obtained were adjusted to a concentration of 5.0×10^8 cells/ml with normal saline.

Determination of immunomodulatory activity

Sprague–Dawley rats were put into six groups with six rats in each group. Animals in all groups were immunized with 5.0×10^8 SRBC/ml after which each group was treated with either STE (37.5, 75 or 150 mg/kg p.o., daily) based on preliminary investigations, levamisole (10 mg/kg, p.o, daily), dexamethasone sodium (4 mg/kg, i.m., daily) or vehicle.

Delayed type hypersensitivity response

On day 15, the delayed type hypersensitivity (DTH) response was measured as described by Saïke *et al.*,^[3] with modifications. The right hind paw thicknesses of the rats were measured. They were then antigenically challenged by administering 2.5×10^7 SRBC /ml, s.c., into the right hind foot pad. The paw thicknesses were again measured after 24 h of challenge and the increase in footpad swelling determined. The increase in paw thickness was used as an index of DTH.

Hemagglutinating antibody titer

On Day 20, the hemagglutinating antibody (HA) titer was determined following the procedure reported by Nelson^[4] with modifications. Blood samples were collected from retro-orbital plexus into test tubes placed on ice. After 1 h, the blood samples were centrifuged to obtain the serum. Ten serial dilutions of the serum in 0.15 M phosphate buffer saline (PBS) pH 7.2 were made and 50 µl of these were then titrated with 25 µl of 2.5×10^7 SRBC /ml. The test tubes were then incubated at room temperature for 2 h and examined visually for agglutination. The reciprocal of the highest dilution of serum showing 50% agglutination is expressed as HA titer.

White blood cell differential count

On Day 28, blood samples obtained from the rats were analyzed for the white cell profile at the Hematology Department, Komfo Anokye Teaching Hospital, Kumasi using the CELL-DYN 1800 auto analyzer. The mean of the results obtained was recorded for each treatment group.

Erythropoietic effect

Basal RBC count and hemoglobin concentration of blood were determined for Sprague–Dawley rats. Five of these were put into a group (Group A- normal rats). Anemia was induced using phenylhydrazine (PHZ) (60 mg/kg, i.p., in divided doses daily, for three consecutive days). Anemia was considered induced when RBC level as well as hemoglobin

concentration of the blood reduced by about 30%. Anemic rats were put into groups B–F with five per group and treated as follows: (Group B-anemic without treatment, C- Ferroglobin 0.15 mg/kg, D, E and F- 37.5, 75, and 150 mg/kg of *Solanum torvum* extract (STE) respectively daily). The RBC number and hemoglobin concentration were determined using the CELL-DYN 1800 auto analyzer every three days for 24 days.

Statistical analysis

GraphPad Prism Version 5.0 for Windows (GraphPad Software, San Diego, CA, USA) was used for all statistical analyses. Data are presented as mean ± SEM and analyzed by one-way ANOVA followed by Bonferroni's multiple Comparison test (post test); $P \leq 0.05$ was considered statistically significant in all analyses. The graphs were plotted using Sigma Plot for Windows Version 11.0 (Systat Software Inc., Germany).

RESULTS

Immunostimulatory activity

The DTH response increased very significantly in groups treated with Levamisole ($P < 0.01$), and 75 and 150 mg/kg/day *S. torvum* ($P < 0.001$) relative to the 'no treatment' group (control). The DTH response for the dexamethasone-treated group decreased significantly ($P < 0.05$) [Table 1]. Levamisole and *Solanum torvum* treatment resulted in significant increases ($P > 0.001$) in the HA titer and WBC count relative to the dexamethasone and 'no treatment' groups [Table 1]. A differential count performed indicated an increase in the neutrophil proportion of the total count in the *Solanum*-treated groups (75 and 150 mg/kg/day)

Erythropoietic effect

After induction of anemia, the number of RBCs and the hemoglobin concentration decreased by 58.73% and 64.98% respectively. There was no significant increase ($P > 0.05$) in the number of RBCs and hemoglobin concentration of the anemic and untreated rats during the experimental period. Treatment of PHZ-induced anemic rats with the reference hematonic (0.15 ml/kg),

and *Solanum torvum* (37.5-150 mg/kg) resulted in significant increase ($P < 0.001$) in both, the number of RBCs and hemoglobin concentration as compared to the untreated PHZ-induced anemic rats [Figures 1 and 2]. Difference between treatment groups was however insignificant ($P > 0.05$). Area under the curve (AUC) values for these are as shown in the Table 2. As the anemic condition improves the AUC value increases.

DISCUSSION

The extract significantly and dose-dependently increased the DTH response, which is a direct correlate of cell-mediated immunity (CMI). A similar effect was observed for levamisole. DTH is a Type IV reaction characterized by large influxes of non-specific inflammatory cells, particularly macrophages leading to the activation of sensitized T_{DTH} cells. Activation of T_{DTH} cells by antigens results in the

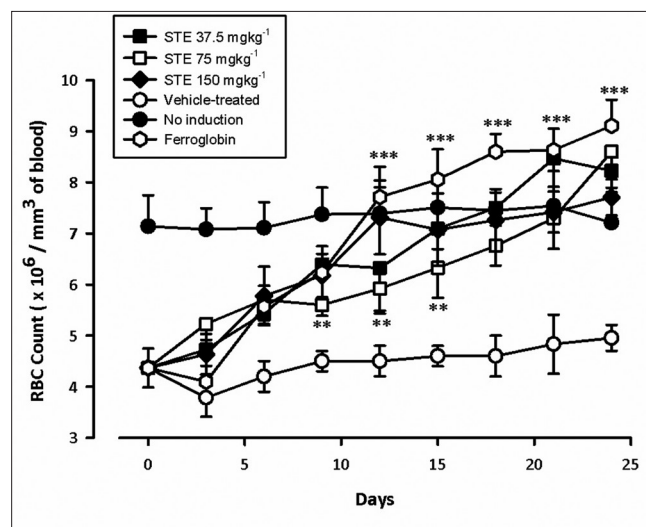


Figure 1: The relationship between the red blood cells count (per mm³ of blood) and time (days) for normal Sprague-Dawley rats, rats in whom anemia has been induced with Phenylhydrazine but not treated, and those in whom anemia has been induced and treated with either a reference hematonic, or three different doses of *Solanum torvum* extract. Values are means ± s.e.m. (n=6). ** $P < 0.01$, *** $P < 0.001$ compared to vehicle-treated group (Two-way ANOVA followed by Bonferroni's *post hoc* test)

Table 1: Details of the results on delayed type hypersensitivity response, hemagglutinating antibody titer, and white blood cells count

Treatments	HA titer	DTH response	WBC count K/ μ L
No treatment (Control)	693.3 ± 314.6	77.67 ± 2.52	8.20 ± 0.36
Levamisole (10 mg/kg/day)	3413 ± 1322*	89.22 ± 1.36**	11.90 ± 0.35***
Dexamethasone (4 mg/kg/day)	1227 ± 768.4 ^{ns}	54.92 ± 2.93 ^{†††}	7.8 ± 0.67 ^{ns}
STE (37.5 mg/kg/day)	2133 ± 661 ^{ns}	85.8 ± 5.65*	12.5 ± 0.66***
STE (75 mg/kg/day)	4053 ± 1701**	121.7 ± 4.32***	14.1 ± 0.81***
STE (150 mg/kg/day)	8533 ± 2644***	181.5 ± 3.29***	15.6 ± 0.57***

For significant increases: ***implies $P < 0.001$, **implies $P < 0.01$; * implies $P < 0.05$; ns implies $P > 0.05$. ^{†††}implies a significant decrease ($P < 0.001$), DTH = Delayed type hypersensitivity HA = Hemagglutinating antibody, WBC = White blood cells, All values are mean ± SD of six rats in each group

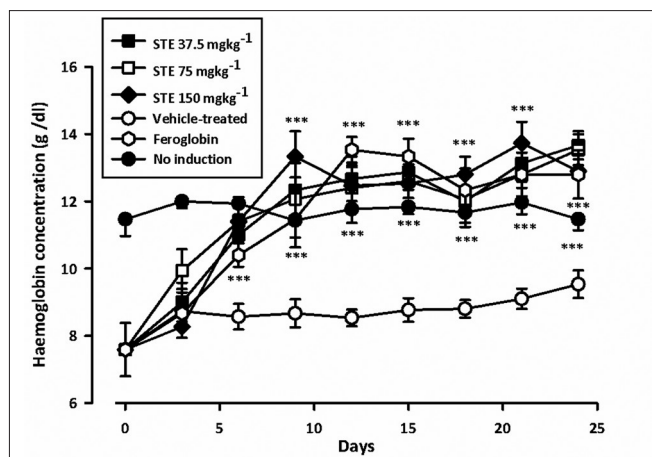


Figure 2: The relationship between the hemoglobin concentration (g/dl) and time (days) for normal Sprague-Dawley rats, rats in whom anemia has been induced with Phenylhydrazine but not treated, and those in whom anemia has been induced and treated with either a reference hematinic, or three different doses of *Solanum torvum* extract. Values are means \pm s.e.m. (n=6). ** $P < 0.01$, *** $P < 0.001$ compared to vehicle-treated group (Two-way ANOVA followed by Bonferroni's *post hoc* test)

secretion of various cytokines including interleukin-2, interferon- γ , macrophage migration inhibition factor and tumor necrosis factor- β ^[5] and subsequent phagocytic activity. There is evidence to suggest that DTH reaction is important in host defense against parasites and bacteria that can live and proliferate intracellularly.^[6] Treatment of STE enhanced DTH reaction, which is reflected from the increased footpad thickness compared to the control group suggesting heightened infiltration of macrophages to the inflammatory site. This study may support a possible role of STE in promoting cell-mediated immune response. The DTH response for the dexamethasone-treated group decreased significantly ($P < 0.05$) as expected of immunosuppressants.

The *Solanum torvum* extract and levamisole significantly increased the HA titer compared to dexamethasone and the control groups. The HA titer was determined to establish the humoral response against SRBC by STE. An increase in HA titer indicates that there has been a proliferation of antibodies (a large dilution of the serum still contains enough antibodies to engage in antigen-antibody reaction) which enhances or boosts the immune system. At neutral pH, RBCs possess a negative ions' cloud that makes the cells repel one another, this repulsive force is referred to as zeta potential. Because of its size and pentameric nature, immunoglobulin M (IgM) can overcome the electric barrier and get cross-link RBCs, leading to subsequent agglutination. The smaller size and bivalency of immunoglobulin G (IgG), however, makes them less capable of overcoming the electric barrier. This characteristic may account for IgM being more effective than IgG in agglutinating red blood cells.^[7] STE treatment

Table 2: The area under the curve values obtained from curves of the mean red blood cell counts, and the mean hemoglobin concentration against time for different treatment groups

Groups	AUC values for curves obtained for	
	Mean RBC count	Mean Hb concentration
Normal Rats	175.9	282.2
Reference drug-treated	166.9***	278.2**
37.5 mg/kg <i>Solanum</i> -treated	156.7**	280.9**
75 mg/kg <i>Solanum</i> -treated	147.9*	281.5**
150 mg/kg <i>solanum</i> -treated	155.1**	284.3**
No-treatment group	103.9	207.2

** $P < 0.01$, *** $P < 0.001$ compared to "No-treatment" anemic group (One-way ANOVA followed by Newman-Keul's *post hoc* test), AUC = Area under the curve, RBC = Red blood cell

improved the HA titer reflecting an overall elevation of humoral immune response

The extract and levamisole treatment resulted in significant increases ($P > 0.001$) in the WBC count relative to the dexamethasone and 'no treatment' groups. A differential count performed indicated an increase in the neutrophil proportion of the total count in the *Solanum*-treated groups. The augmentation of the humoral response as evidenced by an enhancement of antibody responsiveness to SRBC as a consequence of both pre- and post-immunization drug treatment indicates the enhanced responsiveness of macrophages and B-lymphocyte subsets involved in antibody synthesis. Since neutrophils account for 50-70% of WBCs, it can be said that the neutrophil production increased with increasing white cell production. The neutrophils provide the major defense against pyogenic (pus-forming) bacteria and are the first on the scene to fight infection. This suggests that the innate immunity was enhanced by *Solanum torvum*. This effect may probably be due to its high vitamin B complex and vitamin C content since vitamins are known to boost the body's immune system.

STE and the reference hematinic caused a steady and dose-dependent increase in the number of RBCs and hemoglobin concentration in the PHZ-induced anemic rats in comparison to the untreated anemic rats. Phenylhydrazine induces hemolysis of RBCs by inducing the formation of toxic, free radicals that can attack cellular macromolecules like hemoglobin resulting in oxidative damage within the RBCs resulting in their destruction.^[8,9] Again, an attack by free radicals accelerates the normal aging process of the red cells causing premature splenic sequestration. This results in a quantitative deficiency of circulating RBCs and hence hemoglobin.^[10] There was no significant increase ($P > 0.05$) in the number of RBCs and the hemoglobin concentration of the anemic

but untreated rats during the experimental period. Tissue hypoxia which could be caused by a quantitative deficiency of circulating RBCs and hemoglobin was not enough to bring the RBC number and hemoglobin concentration back to the original levels (as was observed in the rats which were not treated after induction of PHZ-anemia). Tissue hypoxia stimulates the erythropoietin-producing cells in the kidney to produce erythropoietin which is a hormone that regulates the proliferation and differentiation of hematopoietic progenitor cells in the bone marrow of all the anemic rats.^[11] This results in the correction of anemia provided that the bone marrow response is not impaired by red-cell nutritional deficiency (especially iron deficiency).

The reference hematinic contains iron which forms the nucleus of the iron-porphyrin haem ring and together with globin chains forms hemoglobin. The vitamin B complex vitamins act as precursor in the synthesis of cofactors for hematopoiesis and protein synthesis.^[11] *Solanum torvum* (also known as vitamin B complex fruit) has as part of its chemical constituents, steroids, glycosides, saponins, fixed oils, vitamin B group, and iron salts.^[1] This may account for its ability to reverse anemia comparable to that of the reference hematinic. *S. torvum* also contains vitamin C which may help in the reduction of iron in the ferric state to the ferrous state resulting in the rapid absorption of iron which will increase hemoglobin synthesis.^[12] The exact mechanisms by which the extract exhibited the reported effects need further mechanistic investigations.

CONCLUSION

The aqueous extract of *Solanum torvum* possesses immunomodulatory and hematinic properties thus supporting its traditional uses as a hematinic and as food for the patients with reduced immunity.

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