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Immuno stimulant activity of *Heterostemma tanjorense* (Wight & Arn) on azathioprine induced male albino rats

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ABSTRACT

Immunostimulators are substances that stimulate the immune system by increasing the activity of any of its individual component biological systems. The selected plant species *Heterostemma tanjorense* Wight &Arn is an area specific medicinal plant. Literature review made on this species reveals not much of its medicinal activity to be explored and documented. The present study reports the immune stimulant activity of *Heterostemma tanjorense* on azathioprine administered male albino rats. Biological parameters such as RBC, WBC, neutrophils, neutrophil adhesive and immunoglobulins were evaluated and the results were reported.The results obtained were in significant quantities and in reportable amounts.

INTRODUCTION

Reduced Immune system activity is responsible for various diseases such as arthritis, ulcerative colitis, asthma, allergy, parasitic and infectious diseases, while defective immune mechanisms are the main cause for diseases such as diabetes mellitus, cancer, myocardial diseases, cirrhosis and atherosclerosis (Samter et al., 1971). These difficulties can be overcome by boosting the immune system using selective immune-stimulant drugs (Ragap, 2012). Azathioprine is an immuno suppressant drug used during organ transplantation and in autoimmune diseases such as rheumatoid arthritis and pemphigus or inflammatory bowel disease such as Crohn syndrome and ulcerative colitis (Gillett and Chan, 2000). It is a prodrug, which in the body is converted to the active metabolite 6-mercaptopurine and 6-thioiosinic acid. Azathioprine act by inhibiting the purine synthesis necessary for the proliferation of cells, especially *leucocytes and lymphocytes* (Konstantopoulou, 2005).

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Immunostimulators are substances that stimulate the immune system by increasing the activity of any of its component systems. An example for such a stimulator include granulocyte macrophage colony stimulating factor (Soehnlein, 2008). Immuno stimulators belong to any one of the following two categories (Antony et al., 2005), viz. 1-Specific immuno stimulators-vaccine or any antigen 2-Non-specific immuno stimulators-adjuvant female sex hormones (Azuma and Jolles, 1987).

More attention is now being given to certain new synthetic immuno stimulators and to their clinical applications in cancer and infectious diseases. The use of plants for medicinal purposes has a very long and unbroken history in the Indian subcontinent (Sumalatha et al., 2012). Natural products, especially the plants used in "Indian traditional medicine" are the potential source of such immunostimulatory compounds. The present study aims at evaluating the aqueous extract of *Heterostemma tanjorense* Wight &Arn for immuno stimulant activity with a view of developing leads for new therapeutic products. The main biochemical constituents of *Heterostemma tanjorense* Wight & Arncomprise flavonoids, triterpenoids, tannins, steroids and anthroquinone. They are used in ophthalmopathy, orchitis, cough, burning sensation, stomachalgia, consumption, fever and tridosa (Thevasundari and Rajendran, 2012).

MATERIALS AND METHODS

Male albino rats weighing between 150-200 g were used for this study. Animal handling and experimental procedures followed were all approved by the Institutional Animal Ethical committee. The animals were kept under a 12-h light/dark cycles at 22°C and 60% humidity along with food and water ad libitum.

Collection of plant

The medicinally viable parts of *Heterostemma tanjorense* Wight &Arn was collected from Herbal Garden, STET Women's College,Mannargudi, which were carefully examined and identified with the help of regional Floras (Nair and Henry, 1987). Specimens were further confirmed with reference to Herbarium sheets available in the Botanical survey of India, (CSIR), Coimbatore, Tamilnadu.

Extraction of plant material

Medicinally useful aerial parts were collected from the field and dried in shade for a period of ten days. The collected parts of this plant were processed, dried in shade and powdered by pulverizes.

Experimental protocol

The male albino rats chosen for the study were divided in to six groups and each group was experimented as follows,

Group I: Rats of this group were treated as control and were administrated with 0.9% normal saline once in a day for a total period of 21 days.

Group II: Rats of this group were administrated with 10 mg dose of azathioprine per day from the first day up to the 21st day.

Group III: Rats of this group were administrated with 10 mg of azathioprine once in a day, from the 1stto the 7th day and were administered with an aqueous extract of 50 mg plant powder of *Heterostemma tanjorens e*Wight &Arn from the 8th to the 21st day at an interval of 24 hours.

Group IV: Rats of this group were administrated with 10 mg of azathioprine once in a day, from the 1stto the 7th day and were administered with an aqueous extract of 100 mg plant powder of *Heterostemma tanjorense* Wight &Arn from the 8th to the 21st day at an interval of 24 hours.

G**roup V:** Rats were administrated with a 10 mg dose of dry powder of azathioprine from the 1st to the 7th day, and an aqueous extract of 150 mg/kg from 8thto the 21stday at an interval of 24 hours.

Group VI: Rats were administrated with 10 mg dose per day of azathioprine from the 1stto the 7th day and were administered with 200mg plant powder of *Heterostemma tanjorense* Wight & Arn in water from the 8thto 21st day at an interval of 24 hours.

At the end of the experimental period of 3 weeks (i.e., on 22nd day), all rats from each group were sacrificed by cervical decapitation and various biochemical parameters were analyzed. Fresh blood was immediately collected by cardiac puncture in fresh sterilized tubes, allowed to clot and the serum was separated by centrifugation at 2500rpm for 15 minutes. The sterile, hemolysis-free serum samples were subjected to biochemical investigation. Hematological parameters, namely the red blood cells (RBC) count and white blood cells (WBC) count was measured by standard procedures (D'Amour et al., 1965). Estimation of Neutrophils adhesive was done by Leishman's staining method (Kajaria, 2013). Proteins were estimated by Lowry's method (Lowry et al., 1951). To detect the immunoglobulins IgG, IgM, IgA, agarose gel electrophoresis was performed (Araki, 1958).

Results

Herbal preparations are becoming increasingly popular for a variety of diseases and infections, primarily influencing the host defense mechanism (Yadav, 2011). Immuno stimulating agents of plant and animal origin enhance the immune responsiveness of an organism against a pathogen by activating the immune system (Patil et al., 1998). Generally animals act as good models for numerous experiments in the field of pharmacology (Löscher, 2011).The selected medicinal plant, *Heterostemma tanjorense* Wight & Arn. Has been identified to possess immuno stimulant activity against Azathioprine administered rats. The results of the study made are revealed.

Haematological parameters

RBC (Table-1)

Among the experimental group of albino rats, those rats that were administered with azathioprine were found to show lower RBC than all the remaining groups (1.05 ± 0.11 million/µl). But in the herbal drug administered group of rats the RBC profile shows a gradual change upon change in the concentrations of plant extract supplied (5.22 ± 0.56d, 5.48 ± 0.34 million /µl). The RBC count in the group of rats were the dosage was maximum (200 mg) was close to that measured in the control group of rats(3.51 ± 0.49 million /µl).

WBC (Table-1)

Results of the WBC count reveal that their content was less in rats when administered with azathioprine (3.28 ± 0.34 thousands /µl). WBC profile was profoundly elevated when the selected plant powder and extract were given to the test groups. (group III& group IV) and at still more high concentrations of drug WBC raised close to normal.(4.51 ± 0.76 thousands /µl).

Table 1: Hematological profile of albino rats on administering*Hetrostemmatanjorense* Wight &Arn.

S.No.	Animals	RBC (Million/cubic mm.)	WBC (Thousand/ cubic mm.)	Protein (mg/dl)	Neutrophils (Thousand/ cubic mm.)	Neutrophil adhesive (%)
1.	Group -1	3.29 ± 0.32	5.27 ± 0.67	5.43 ± 0.36	23.57 ± 0.27	33.56 ± 0.23
2.	Group II	1.05 ± 0.11[d]	3.28 ± 0.34[a]	3.02 ± 0.61[d]	12.40 ± 0.51[a]	21.31 ± 0.21
3.	Group III	5.22 ± 0.56[d]	8.67 ± 0.37[a]	5.29 ± 0.94[d]	15.13 ± 0.67[a]	22.35 ± 0.16
4.	Group IV	5.48 ± 0.34[d]	8.40 ± 0.40[a]	4.95 ± 0.88[d]	15.39 ± 0.56[a]	25.41 ± 0.25
5.	Group V	2.42 ± 0.20	5.97 ± 0.69	5.39 ± 0.96	23.40 ± 0.23	27.37± 0.22
6.	Group VI	3.51 ± 0.49	4.51 ± 0.76	5.53 ± 0.84	22.14 ± 0.20	30.18 ± 0.21

The results expressed are mean ± SD; n = 3., a -Significant change against control (P < 0.050)., d- Significant change against control (P < 0.001)

Protein (Table- 1)

The results of the protein analysis in the test animals reveal that the protein production is decreased in rats with azathioprine administration and there was also less production in cases with low drug concentrations, but the protein profile shows an increase when the drug levels goes high (5.53 ± 0.84 mg/dl).

Neutrophil (Table-1)

Neutrophil content of experimental groups has exhibited varied responses. Comparing with the normal rats, those animals fed with azathioprine had less neutrophil production i.e., almost reduced to one half, but this started increasing when the selected medicinal plant drug was given at high doses.

Group of rats from II to VI neutrophil amount was observed to risegradually almost close to normal level especially at a dose of 200 mg herbal drug (22.14± 0.20 thousands /µl).

Neutrophil adhesive (Table-1)

Among all the groups, those groups of rats that were administered with high drug concentrations gave high neutrophil adhesive (30.18±0.21%). In rats that were administered with azathioprine and in rats given with 50 mg dosage of drug there was a raise in neutrophil adhesive content.

Immunoglobulin (Table 2)

Immunoglobulin G levels reduced in the experimental rats due to the administration of Azathioprine. Normal rat were measured to produce 860mg/dl to 515mg/dl. Ig G levels kept decreasing on increasing the drug quantity and become equal at 200 mg concentration.

Table 2: Immunoglobulin (IgM, IgA and IgG) levels in test rats with *Hetrostemmatanjorense* Wight &Arn

S.No.	Animals	Ig G (mg/dl)	Ig A (mg/dl)	IgM (mg/dl)
1	Group -1	860	172	7.8
2	Group II	515	102	3.4
3	Group III	582	129	4.4
4	Group IV	632	137	5.1
5	Group V	790	160	6.8
6	Group VI	840	173	7.7

The same trend in drug action was observed in Ig M levelsls also. It is to note that the quantity of immunoglobulins decreased when azathioprine was administered. A regular trend in drug action could be identified when the drug concentration was gradually raised with respect to IgA levels.

DISCUSSION

Immunostimulant agents of plant and animal origin enhance the immune responsiveness of an organism against a pathogen by activating the immune system (Sathianarayanan, 2012). However these agents and the herbal formulations should be subjected to systematic studies to substantiate the therapeutic claims made with respect to their clinical utility (Kulkarni and Karande, 1998).

In this study, the hematological parameters exhibited results that supported the immunostimulant activity ofthe medicinal plant *Heterostemma tanjorense Wight* &Arn..Earlier also hematological parameters are the supporting evidences for the immunostimulant activity in neem bark (Vander Nat et al., 1987). Recently, an aqueous extract of stem bark has been shown to enhance the immune response of Balb-c mice to sheep red blood cells *in vivo* (Njiro *et al.*, 1999). The aqueous extract of leaf also possesses potent immunostimulant activity as evidenced by both humoral and cell-mediated responses (Sen *et al.*, 1992). Neem oil has been shown to possess immunostimulant activity by selectively activating the cell-mediated immune mechanisms to elicit an enhanced response to subsequent mitogenic or antigenic challenge (Upadhyay *et al.*, 1992).

Immunosuppressive conditions were induced by the administration of Azathioprine. Highly elevated or reduced conditions of biochemical compounds gave significant results due to the administrations of *Heterostemma tanjorense* Wight & Arn (at doses of 50, 100, 150 200 mg/kg). Higher concentrations of selected drug activity were very effective, which was nearby the normal values of RBC and WBC. Neutrophil adhesive factorsand neutrophil index also have remarkable changes in the experimental groups. Highly decreased amount of protein level was increased by the administration of higher dose of plant extract.

In this study, suppressed amount of immunoglobulins were recorded from the administration of Azathioprine, which was gradually increased due to the administration of this plant product. Normal albino rat containing IgG range from 86mg/dl to 515mg/dl. The percentage was higher in groups administered with higher doses. 150mg/rat administered has higher amount of immunoglobulin A (148mg/dl) and IgM was 5.6mg/dl in group IV. Same levels of immunoglobulin status were recorded in the study of leaf extract at 100 mg/kg after three weeks of oral

administration. There are reports stating that administration causes higher IgM and IgG levels along with increased titre of antiovalbumin antibody (Ray et al., 1996).

Heterostemma tanjorense Wight & Arn has shown significant immunostimulant effect in animals. Therefore clinical and phytochemical screening especially secondary metabolic compounds can lead to discovery of new drug.

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