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RESEARCH ARTICLE

EVALUATION OF IMMUNOMODULATOR Y ACTIVITY OF HYDRO-ALCOHOLIC EXTRACT OF LEAVES OF CESTRUM NOCTURNUM IN WISTAR RATS

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ABSTRACT

Objective: To investigate immunomodulatory activity of hydro-alcoholic extract leaves of Cestrum nocturnum in wistar rat. ***Method:*** Cestrum nocturnum leaves extract in hydroalcoholic solution were prepared by Soxhlation method for 8 hrs at 55-60^{0}C and stored at 22^{0}C in a sealed airtight container. Hydro-alcoholic leave sextract of Cestrum nocturnum was screened for immunomodulatory activity and given to the wistar rat at a concentration of 200 mg/kg and 400 mg/kg of body weight in different groups of 6 mice each orally once a day for 14 days. Levamisole is also given to another group to support the result at a dose of 50mg/kg of body weight orally once a day for 14 days. DTH, HA, TLC, DLC are calculated for the rats. ***Results:*** Oral administration of the extracts for 14 days caused a significant (5< 0.01) reduction of paw edema by using the extract in different concentration, by the Delayed hypersensitivity reaction. Cellular events finally result in increased production of cytokines viz. IL-2, IL-6, IL-12, IFN-γ and TNF-α10-12. It is established that, IL-12 and TNF-α plays a vital role in both innate and adaptive immunity. present experimental findings with DTH and HA titer clearly demonstrated that the treatment of Cestrum nocturnum enhanced the proliferation of T cell and B-lymphocytes, ultimately leading to improvement of both the arms of immunity. The extract also improved other altered biochemical parameters associated with immunity. Also, the changes in food intake, water intake, and weight of internal organs were also restored to normal by the prolonged effect of extract treatment.

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INTRODUCTION

All through human history, vast arrays of natural compounds, particularly those from plant sources, have provided a wealth of immunomodulator. In many ways, they help preserve all life forms and their relations, even in the midst of adversities and mutual antagonisms[1]. Ayurveda, Siddha and Unani are the branches of systems in medicine that provides health care to a large part of population of India. Of these three systems of medicine Ayurveda is the most ancient systems of medicine today. The term Ayurveda, a Sanskrit word, comprises of two parts ayur (life) and veda (knowledge). The ancient Ayurvedic medicinal system was highly developed, and many have considered it to be the first medicinal system. Sushruta was probably the first doctor to practice and teach surgery. In recent times, an interest in natural remedies, including Ayurveda, has been reawakened[2]. The plant kingdom is a treasure house of potential drugs. Drugs from the plants are easily available, less expensive, safe and efficient and rarely have side effects. According to World Health Organization (WHO), medicinal plants would be the best source to obtain variety of drugs. However, plants should be investigated to better understand their properties, safety, and efficiency. Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids. These compounds are synthesized by primary or rather secondary metabolism of living organisms. Plant products have been part of phytomedicines since time immemorial. This can be derived from barks, leaves, flowers, roots, fruits, seeds[3]. Polysaccharides have drawn increasing attention from researchers and consumers, due to their obvious antitumor, antioxidant, anti-HIV/AIDS and immunostimulatory activities. Therefore, the discovery and evaluation of polysaccharides with antitumor and immunostimulatory properties has become an important focus of research10. Literature indicates that the herbal antioxidants concurrently exhibit significant immunomodulatory activities. It is therefore of great interest to investigate immunomodulatory effects of herbal polysaccharides that exhibit antioxidant activity with low toxicity[4]. Immunology is a branch of biomedical science that examines the structure, function and all the other aspects of the immune system in all organisms. The earliest concept of immunity was revealed during the plague of Athens in 430 BC. During the 18th century, Pierre-Louis Moreau de Maupertuis made experiments with scorpion venom and observed that certain dogs and mice were immune to the venom. All these functionalities of the acquired immunity were observed and were later exploited by Louis Pasteur in his development of vaccination and his proposed germ theory of disease.

His theory was in direct opposition to contemporary theories of disease, such as the miasma theory. It was not until Robert Koch's 1891 proofs, for which he was awarded a Nobel Prize in 1905.[5,6] Immunotherapy or immunomodulatory activity is the treatment of a malady by creating, progressing or overcoming a resistant reaction. Immunotherapies, created to get or increase a safe reaction, are classified as immunostimulants. On the other hand, immunotherapies arranged to decrease or stifle, are grouped as immunosuppressants.[7] Cell based immunotherapies are shown to be useful for some cancers. Immune effector cells such as lymphocytes, macrophages, dendritic cells, natural killer cells (NKs) and cytotoxic T lymphocytes (CTL), operate concurrently to guard the body toward cancer by marking unusual antigens represented on the surface of the malignant cells due to mutation.[8] The immunomodulating characteristics of plants are being inspected broadly to realize the alluring impacts on infection avoidance. Subsequently, homegrown cures have been utilized for centuries for security, adequacy, minor side impact, and social worthiness. Hence, plants and their products are safe and so, there's the nonstop application of plant items as a discretionary way to remedy the patients and this approach is in polish from old times.[9] Immunomodulatory drugs modify the response of the immune system by increasing (immunostimulators) or decreasing (immunosuppressives) the production of serum antibodies. Immunostimulators are prescribed to enhance the immune response against infectious diseases, tumours, primary or secondary immunodeficiency, and alterations in antibody transfer, among others. Immunosuppressive drugs are used to reduce the immune response against transplanted organs and to treat autoimmune diseases such as pemphigus, lupus, or allergies.[10,11]

Cestrum nocturnum is a garden shrub from the family Solanaceae, commonly known as "lady of the night" which is used as a remedy for different health disorders. This sprawling shrub has glossy simple leaves, vine like stems, greenish-creamy white tubular flowers and fleshy berries. The berries are marfil white or aubergine in colour. The species name ‘nocturnum’ refers to the species’ habit of opening its small, heavily-scented flowers at night. The flowers release powerful sweet perfume at night. It is made into a rare attar (raat ki rani) which is used in Indian and Middle East perfumery. It is said to be the world’s strongest smelling plant. Indeed, the scent can reach up to 165 feet away from the location of plant.[12] The genus name Cestrum is thought to be derived from the Greek word ‘kestron’, for similarity to a plant of that name, or ‘kestrum’, a tool used for engraving, which the plant’s anthers resemble.[13] Like several other members of the Cestrum genus, C. nocturnum is of Neotropical origin. While night blooming jasmine is a gorgeous plant with charming blooms, the scent also produces severe allergic reactions in some individuals. As reported, the immune system is one of our most complex biological systems in the body, the basic role of the immune system is to distinguish self from non-self which could be an infectious organism, a transplanted organ or an endogenous cell that can be mistaken as a foreign body. The immune responses of the human body against any non-self are of two types: (a) Innate (Natural or Non-specific) immune response and (b) Adaptive (or Acquired or Specific) immune response.

METHODOLOGY

Chemicals and Drugs:The Sheep Red Blood Cells (SRBCs) were procured from Local market Shop, Yelahanka. The drug Levamisole (Cipla Pvt. Ltd., INDIA) purchased from a medicine shop in Yelahanka and all the chemicals were purchased from SD Fine Chemical Limited in Aditya Bangalore Institute of Pharmacy Education and Research, Yelahanka, Bengaluru, Karnataka.

Collection and authentication: The leaves of *Cestrum nocturnum*, was selected for investigation and were procured from the nearest area of Yelahanka, Bengaluru during the time of December 2021. The plant material was taxonomically identified and authenticated by Dr. K. MADHAVA CHETTY, M.Sc., M.Ed., M.Phil., Ph.D., PG DPD, Plant Taxonomist (IAAT:357), Assistant Professor, Department of Botany, SRI VENKATESWARA UNIVERSITY, Tirupathi – 517 502, Andhra Pradesh, INDIA.**(Voucher no: 0956, dated: 02.06.2022)**

Preparation of plant extract: The fresh leaves were air dried in shade and extracted with water and alcohol in a ratio of 50:50 for hydroalcoholic extract, using a Soxhlet extractor for 8 hrs. at 55-60^{0}C. The supernatant was filtered through Whatman filter paper No.1 and concentrated under reduced pressure using vacuum at 44 ± 10^{0}C in a rotavapor (IKA ® RB 10 Rota Evaporator, India) The extract was stored at 22^{0}C in a seeded airtight container.

The percentage of extract yield was calculated by using the formula:

% of extract yield= (weight in gm of extract obtained)/ (weight in gm of plant material taken) ×100

Phytochemical Screening: The extract was subjected to phytochemical analysis to test the presence of carbohydrates, glycosides, alkaloids, flavonoids, saponins, phenolic compounds and tannins, proteins, Amino acids, and sterolsin leave extracts.[15]

Acute toxicity study:[14] The acute toxicity study revealed the nontoxic nature of all the extracts even at a higher dose of 4 g/kg body weight of rat for oral route of administration. For the present study the dose is being selected as 500mg/kg *p.o.*

Animals: Wistar rats (average body weight 150-200g), used from in house laboratory. The animals were maintained under standardized environmental conditions (22-28^{o}C, 60-70% relative humidity, 12-hour dark/light cycle) in animal house, Department of Pharmacology, Aditya Bangalore Institute of Pharmacy Education and Research, Yelahanka, Bengaluru, Karnataka. The animals were provided with standard mouse chow (Sai Durga Feeds and Foods, Bangalore, India) and water ad libitum. Experimental protocols and procedures used inthis study were approved by the Institutional Animal EthicsCommittee of Aditya Bangalore Institute of Pharmacy Education and Research, Yelahanka, Bengaluru, India. (Project Proposal No: -61/1611/CPCSEA).

Experimental Protocol:

24 rats were divided into four groups of six animals each.

Group-I: Control – Vehicle *p.o* (Normal Saline 0.9% w/v).

Group-II: *Cestrum nocturnum* hydroalcoholic extract was administered at a dose 200mg/kg/day by oral route for 14 days.

Group-III: *Cestrum nocturnum* hydroalcoholic extract was administered at a dose of 400mg/kg/day by oral route for 14 days.

Group-IV: Standard – Levamisole was administered at a dose of 50mg/kg/day by oral route for 14 days.

Statistical Analysis: All values of results are presented asmean ± standard error ofmean (SEM).The statistical analysisinvolving two groupswhereas one way analysis of variance (ANOVA) followedby Dunnett’s multiple comparison posttest was used forstatistical comparison between control and various treatedgroups. Statistical significance was accepted at the $P< 0.05$values.

Experimental setup: The animal model is required to study the [1] Delayed type hypersensitivity (DTH) reaction[15], [2] Humoral antibody (HA) titer[16], [3] Total leukocyte count (TLC)[17], [4] Differential leukocyte count (DLC)[18].

RESULTS

Preliminary phytochemical screening of hydroalcoholic leaf extract of Cestrum nocturnum shows the presence of secondary metabolites like Alkaloids, Carbohydrates, Proteins, Flavanoids, Saponins, Cardiac glycosides, Tannins, Steriods and percentage of yield of the extract was 13.12%.

Table 1. Effects of Test Extracts and Standard Drug on DTH Response in Rats Using Sheep's RBCs as Antigen

Group	Treatment	Dose	DTH Response (mm) mean paw edema
I	Control	---	2.48±0.235
II	TestextractI	Cestrum nocturnum 200 mg/kg	3.25±0.2658*
II	TestextractII	Cestrum nocturnum 400mg/kg	4.53±0.2416**
III	Standard	Levamisole 50mg/kg	4.71±0.1560***

Table No 2. The effect of test extract and standard drugs on the Humoral Antibody Titer in wistar rats

Group	Treatment	Dose	Antibody titer means ±sem
I	Control	---	9±1.897
II	TestextractI	Cestrum nocturnum 200 mg/kg	339.57±76.43*
II	TestextractII	Cestrum nocturnum 400mg/kg	417±138.65**
III	Standard	Levamisole 50mg/kg	452±132.14***

Table 3. Total leukocyte count

Sl. No.	Group	Mean Leukocyte count
1	Control	5.01×10^3/cu.mm±0.296
2	Cestrum nocturnum200 mg/kg body weight	6.89×10^3cu.mm±0.248*
3	Cestrum nocturnum400 mg/kg body weight	9.41×10^3cu.mm±0.31**
4	Levamisole 50 mg/kg	14.6×103cu.mm±0.138***

Dunnett test and p values as significant* if p<0.05, highly significant** if p<0.01, and extremely highly significant*** if p<0.001 as compared to control.

Table 4. Differential leukocyte counts

Group	Treatment	Dose	Mean % of lymphocytes	Mean % of Neutrophils	Mean % of Eosinophils
I	Control	---	26.4±0.916	66.71±0.431	5.21±0.08
II	TestextractI	Cestrum nocturnum 200 mg/kg	29.21±0.431*	69.54±0.42*	5.6±0.141*
II	TestextractII	Cestrum nocturnum 400mg/kg	36.1±0.34**	75.08±0.546**	6.5±0.01**
III	Standard	Levamisole 50mg/kg	37.98±0.685***	79.48±0.71***	7.01±0.483***

Dunnett test and p values as significant* if p<0.05, highly significant** if p<0.01, and extremely highly significant*** if p<0.001 as compared to control

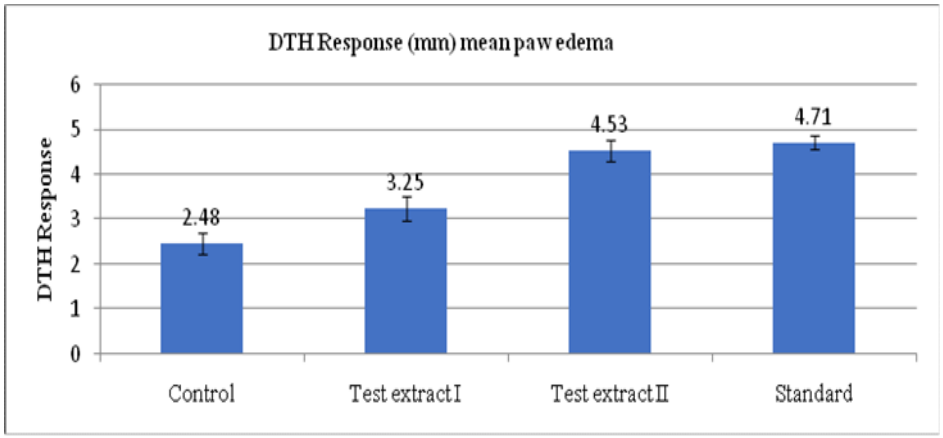
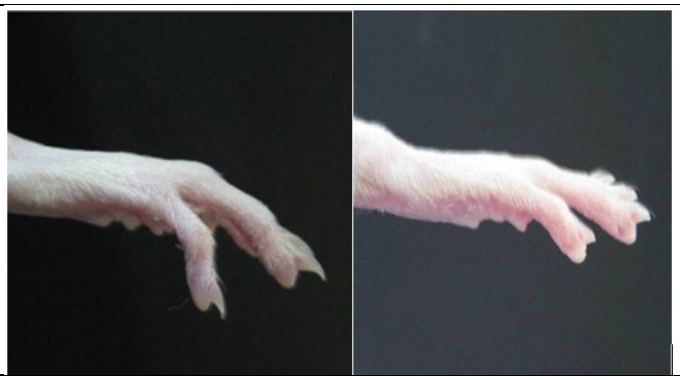
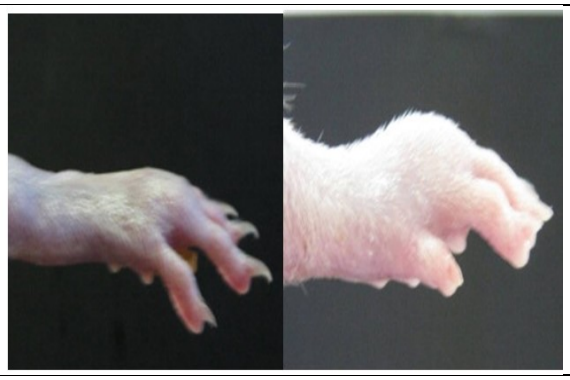


Figure 1. Delayed type hypersensitivity reaction



Sheep's RBC Control Animal *Cestrum nocturnum*200 mg/kg



***Cestrum nocturnum* 400mg/kg Levamisole 50 mg/kg**

Figure 2: Paw edema observed in animals

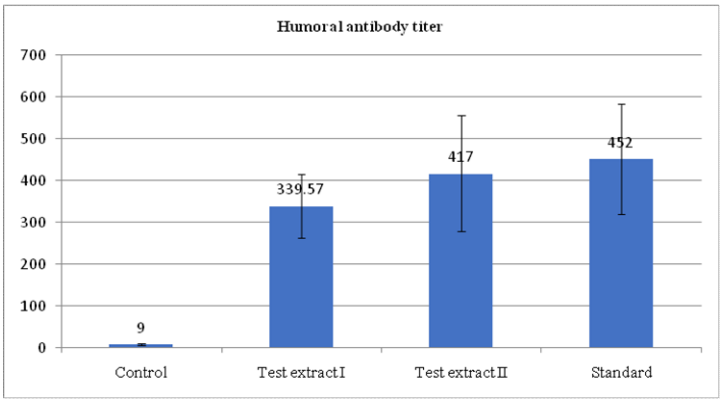
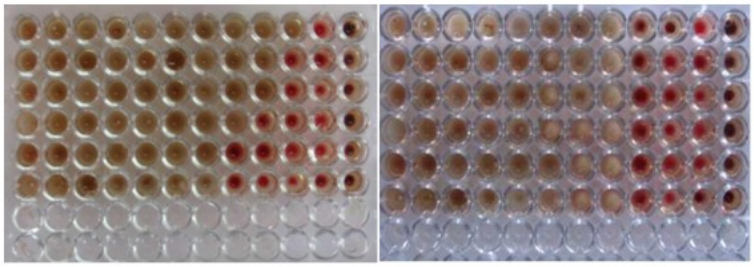
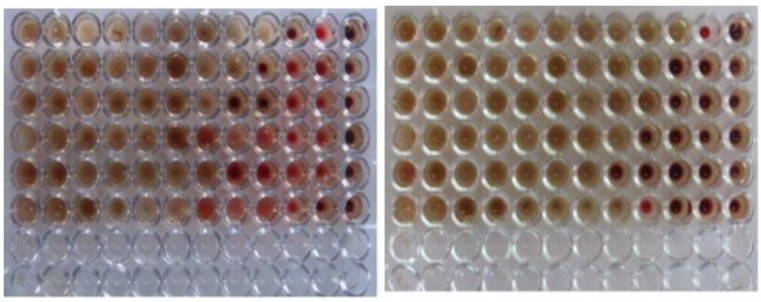


Figure 3. Humoral antibody titer



Sheep's RBC Control Animal Cestrum nocturnum200 mg/kg



Cestrum nocturnum 400mg/kg Levamisole 50 mg/kg

Figure 4. Effect of test extract and standard drugs on the Humoral Antibody Titer in wistar rats

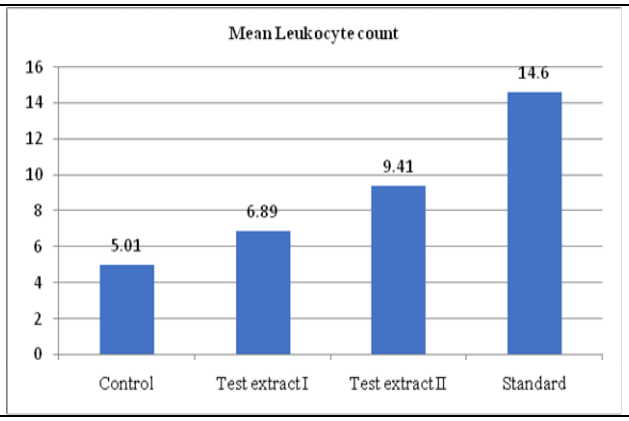


Figure 5. Total leukocyte count

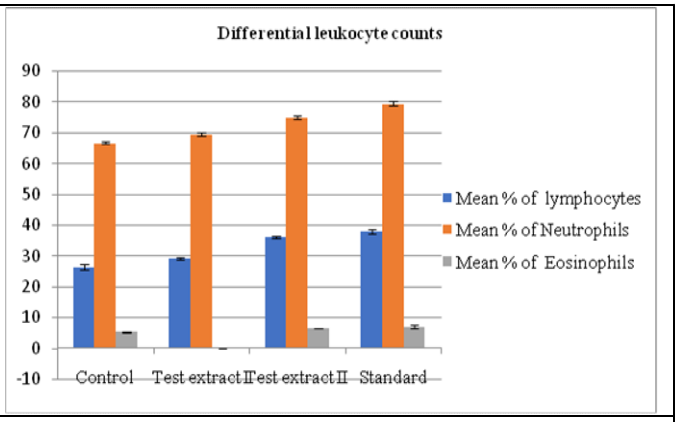


Figure 6. Differential leukocyte counts

DTH Response in Rats Using Sheep's RBCs as Antigen: Effects of Test Extracts and Standard Drug on DTH Response in rats Using Sheep's RBCs as Antigen. The effect of test extract and standard drugs on the DTH response in wistar rats using SRBCs as antigen, administration of hydroalcoholic extract of *Cestrum nocturnum*at the dose of 200mg/Kg and 400mg/Kg and Levamisole 50mg/Kg treatments which were given orally for 14 days showed significant increase in paw edema compared to control group.

The standard drug Levamisole showed the maximum increase in paw edema volume compared to all groups. The results are shown in below table 1.

Mean Humoral Antibody titre: Administration of hydroalcoholic extract of *Cestrum nocturnum* at the dose of (200& 400 mg/kg) and Levamisole 50mg/Kg treatments which were given orally for 14 days

showed highly significant increase in antibody titer values compared to control group. The results are shown in below table 2.

Total Leukocyte Count: The effect of test extract and standard drugs on Total Leukocytes in wistar rats, administration of hydroalcoholic extract of *Cestrum nocturnum*at the dose of (200,400 mg/kg) and Levamisole 50mg/Kg treatments which were given orally for 14 days. The low dose of extract (200 mg/kg) did not show any effect on TLC count compared to control group, whereas the 400mg/Kg and standard drug Levamisole 50mg/Kg showed significant increase in total leukocytes count values compared to control group. The results are shown in below table 3.

Differential Leukocyte Count: The effect of test extract and standard drugs on Differential Leukocytes count in Wistar rats, administration of hydroalcoholic extract of *Cestrum nocturnum* at the dose of (200,400 mg/kg) and Levamisole 50mg/Kg treatments which were given orally for 14 days.

DISCUSSION

Delayed Type Hypersensitivity Response: In this parameter the both lower dose and higher dose of the test (200 mg and 400 mg/kg) had shown significant result in increase in paw edema when compared with control. The standard drug Levamisole had shown the maximum increase in paw volume.

Humoral Antibody Titer: In this parameter both the dose of 200 mg/kg and 400 mg/kg of *Cestrum nocturnum* produced significant result, standard drug Levamisole at a dose of 50 mg/kg also produced significant increase in the titer value.

Total Leukocyte Count: In this parameter the lower dose of *Cestrum nocturnum* 200 mg/Kg had shown no significant increase and higher dose of the hydroalcoholic extract of *Cestrum nocturnum* 400 mg/Kg showed a highly significant increase in the mean total leukocyte count, as compared to control. The results were highly significant for the standard drug Levamisole 50 mg/kg as compare with the test and control group.

Differential Leukocyte Count: For the differential leukocyte count the results revealed for lower dose of *Cestrum nocturnum* showed no significant increase in mean percentage of lymphocytes, Eosinophils and Neutrophils increase in values as compared to control. The results obtained from the animals that received higher dose (400mg/kg) of hydroalcoholic extract, revealed the fact there was a highly significant increase in the mean percentage of lymphocytes and significant increase in the mean percentage of neutrophils respectively when compared to control. The effect of this extract was comparable to the standard drug levamisole all the data represents the Immunostimulatory activity of hydroalcoholic extract of *Cestrum nocturnum*.

CONCLUSION

The results of present study revealed that the hydroalcoholic extract of leaves of *Cestrum nocturnum* generally shown immune stimulatory effect on the humoral immune function and cell mediated immunity in Wistar rats. Further, Studies are required to gain more insights into the possible mechanism of action.

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