Research Article

Assessment of Immunomodulatory activity of *Annona Squamosa* leaves in Pyrogallol induced immunosuppression

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Abstract

Background: The immune system has a fundamental role in protecting the body against pathogenic microbial agents. Environmental pollutants and dietary habits cause disturbances in immune activities and diet containing micronutrients and antioxidants are known to prevent these alterations. **Objective**: The aim of the present study was to investigate the immunomodulatory potential of ethanolic extract of *Annona squamosa* leaves and the extract reported to contain polyphenols, flavonoids and tannins. **Material and methods**: Ethanolic extract of *Annona squamosa* leaves at the doses of 50 mg/kg and 100 mg/kg (per oral) were studied for the assessment of Immunomodulatory activity. To induce immunosuppression, pyrogallol (100 mg/kg, i.p.) was administered. The mice were sensitized with sheep red blood cells (SRBC) to assess the immunological study. In cellular immune response model, SRBC (0.5x109 cells/100g, i.p.) were injected in the sub plantar region of the hind paw. **Results and conclusion:** An increase in paw volume was recorded in pyrogallol-induced immunosuppressed mice. The non-specific immune response was assessed by carbon clearance assay. The Leaf extract of *Annona squamosa* increased the footpad thickness and phagocytic index in carbon clearance assay. From the above findings, it is concluded that ethanolic extract of *Annona squamosa* leaves possesses potential Immunomodulatory activity.

Keywords: *Annona squamosa,* Pyrogallol, immunomodulatory, flavonoids

Introduction

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Many of these isolations were based on the uses of the agents in traditional medicine. The plant-based, traditional medicine systems continues to play an essential role in health care, for about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care (Omonkhelin et al., 2007). Interest in medicinal plants as a re-emerging health aid has been fuelled by the rising costs of prescription drugs in the maintenance of personal health and well being and the bioprospecting of new plant-derived drugs (Lucy and Edgar, 1999). The ongoing growing recognition of medicinal plants is due to several reasons, including escalating faith in herbal medicine. Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of drugs and chemotherapeutics from these plants as well as from traditionally used herbal remedies (Adesokan et al., 2008). According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should be investigated to better understand their properties, safety and efficacy (Doughari et al., 2008).

The immune system is a system of biological structures and processes within an organism that protects against disease. To function properly, an immune system must detect a wide variety of agents, from viruses to parasitic worms, and distinguish them from the organism's own healthy tissue. Pathogens can rapidly evolve and adapt to avoid detection and neutralization by the immune system. As a result,

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multiple defense mechanisms have also evolved to recognize and neutralize pathogens. Even simple unicellular organisms such as bacteria possess a rudimentary immune system, in the form of enzymes that protect against bacteriophage infections. Other basic immune mechanisms evolved in ancient eukaryotes and remain in their modern descendants, such as plants and insects. These

Mechanisms include phagocytosis, antimicrobial peptides called defensins, and the complement system.

Now a day's lot of people suffering from the different disease and disorders, and one of the main is stress, it is directly related to the mind and body, causes significant effect on immune response in general (Roshan and Poojari, 2013). Immunomodulation is the mechanism in which the administered drugs or compound alters the immune response in different manner. Immunosuppressant drugs play important role in autoimmune disease and transplantation of different organs. Drugs which act as immunosuppressant are cyclosporine, Cyclophosphamide, and azathioprine (Priyanka et al., 2012).

Annona squamosa which is commonly called as custard apple, and which grown throughout India, but it is the native of the West Indies and Central America. The pulp contains 58% of sugar having several medicinal properties. *Annona squamosa* which is having different medicinal properties like antioxidant, insecticide, antitumor agent, anti diabetic, anti-inflammatory, due to the presence of cyclic peptide. During dysentery decoction of the leaves are provided, and also used during ulcer and wounds (Gajalakshmi et al., 2011).

This plant parts contain different chemical constituents like saponins, flavanoids, steroids, glycosides, and these are very important for the immunosuppression activity and hence there is a chance of leaves of immune modulatory activity can be carried out by using leaves of *Annona squamosa.*Hence the present study was designed to evaluate the Immunomodulatory activity of leaves of *Annona squamosa.*

Materials and Methods

Preparation of extract and Identification of phytoconstituents

Annona squamosa leaves were collected from local area of Chitradurga. The mentioned parts of plants are were first dried and pulverized to particle size (#)40 and then were first defatted with petroleum ether (40-60^{0}C) and extracted with ethanol by continuous hot percolation method using soxhlet apparatus at 40^{0}c for 48 hr to obtain ethanol extracts of leaves. The filtrates of the extracts were concentrated to dryness at 40^{0}c under reduced pressure in a rota flash evaporator (Sanjeev et al., 2012).

Animals:-Swiss albino mice of either sex, weighing 25-30gm housed in standard conditions of temperature, humidity and light were used. They were fed with standard rodent diet and water ad libitum. The study was approved by Institutional Animal Ethical Committee. IAEC certificate enclosed (Ref.No.01 SJMCP/IAEC/2016-17) Dated 09/03/2017.

Pyrogallol Induced Immunosuppression Method (Vikas et al., 2010)

Animals were divided into five groups of six animals each.

Group I animals served as control and received equivalent volume vehicle, Group II animal are administered with pyrogallol (100mg/kg/ip daily for seven days), Group III animals were given with pyrogallol daily for seven days and vitamin E suspension (150mg/kg p.o.). Group IV and V were receive extract of plants.

Groups	No of animals	Treatment
Group I	Six	Normal Control
Group II	Six	Treated control with pyrogallol 100mg/kg
Group III	Six	Treated standard with Vitamin E suspension 150mg/kg
Group IV	Six	Treated with Plant extract lower dose 50mg/kg
Group V	Six	Treated with Plant extract higher dose 100mg/kg

Immunological parameters

Cellular immune response (foot pad reaction test):-To study the cellular immune response, the edema was induced in the right paw of mice by injecting the Sheep Red Blood Cells (SRBC) (0.025X10^9) in the sub planar region on 20th day, increase in the paw volume on 48 hour, .i.e. on 22nd day assessed on pleythysmometer, the mean percentage increased in the foot volume considered as the delayed type hypersensitivity reaction and as an index of all cell mediated immunity. The volume of the left hind paw, injected similarly with phosphate buffered saline served as control.

Carbon clearance (% phagocytosis)

On last day, 3hours after the last dose all the animals of each group would be injected with 0.1ml of carbon ink suspension (1.6 v/v in 1% gelatin dissolved in saline) through i.v. route via the tail vein. The blood samples (50µl) was taken at intervals of 0 min and 15 min after injection and dissolved in 0.15% w/v disodium EDTA (50µl). 25µl of sample would be mixed with 2ml of 0.1% sodium carbonate solution. Absorbance would be read at 660nm taking 0.1% sodium carbonate solution as blank. The phagocytic index (K) would be calculated using the formula:

$$K = \frac{(\ln OD_1 - \ln OD_2}{(t_2 - t_1)}$$

Table 1. Effect of Ethanolic extract of *Annona squamosa* leaves on Cellular Immune Response in pyrogallol induced immunosuppressed mice

Group (N = 6)	Treatment	Footpad thickness mean difference (mm)
I	Normal Control	0.648 ± 0.037
II	Treated control with pyrogallol 100mg/kg	0.194 ± 0.053***
III	Treated standard with Vitamin E suspension 150mg/kg	0.713 ± 0.056***
IV	Treated with Plant extract lower dose 50mg/kg	0.348 ± 0.053**
V	Treated with Plant extract higher dose 100mg/kg	0.531 ± 0.094***

Values are expressed as Mean ± SEM.;*= p<0.05, **= p<0.01, ***= p<0.001 .Test drug treated groups were compared with control group (Group I).

Table 2. The effect of Ethanolic extract of *Annona squamosa* leaves on Carbon Clearance Assay by Phagocytic Index in treated control mice.

Group N=6	Treatment	Phagocytic Index
I	Normal Control	53.4 ± 3.14
II	Treated control with pyrogallol 100mg/kg	27.3 ± 4 34***
III	Treated standard with Vitamin E suspension 150mg/kg	69.3 ± 5.15*
IV	Treated with Plant extract lower dose 50mg/kg	38.1 ± 5.15*
V	Treated with Plant extract higher dose 100mg/kg	43.6 ± 3.94**

Values are expressed as Mean ± SEM.;*= p<0.05, **= p<0.01, ***= p<0.001 .Test drug treated groups were compared with control group (Group I).

Where, OD_1 and OD_2 are the optical densities at time t_1 and t_2 respectively.

Statistical analysis

The results obtained from the above investigation was Subjected to statistical analysis using one way ANOVA followed by Tukey- Kramer Multiple Comparisons test.

Results

In the present study, the Immunomodulatory activity of ethanol extract of *Annona squamosa* leaves was assayed in Swiss Albino Mice using Cellular immune response (foot pad reaction test) method and Carbon Clearance Assay by Phagocytic Index. Table 1 and 2 shows that the Immunomodulatory activity of ethanol extract of *Annona squamosa* leaves significantly inhibited the Cellular immune response for 50 and 100 mg/kg of ethanol extract of *Annona squamosa* leaves respectively. These results indicated that ethanol extracts with a dose of 50mg/kg body weight and 100mg/kg body weight showed a maximum Immunomodulatory activity which is similar to the Standard drug Vitamin E suspension.

Discussion

The innate immune system is the first line of defense. The major effectors of innate immunity are complement, granulocytes, monocytes/macrophages, natural killer cells, mast cells and basophiles. An intact skin or mucosa is the first barrier to infection. When this barrier is broken bacterial destruction is accomplished by lysozyme which breaks the peptidoglycon cell wall and split the product arising from compliment activation (Katzung, 2001;Davidson‟s, 1999).

Immunomodulatory activity of ethanolic extract of *Annona squamosa* leaves. was explored by evaluating their effects on Pyrogallol induced immunosuppression in mice at 2 dose levels of 50 mg/kg and 100 mg/kg (per oral). Results of the study revealed the dose dependent counteracting effect of the ethanolic extract of *Annona squamosa* leaves. Pyrogallol induced suppression of humoral as well as cell mediated immune response were significantly attenuated by daily oral treatment with ethanolic extract of *Annona squamosa* leaves. Vitamin E treated group exhibited similar attenuation of the suppression in immune responses. *Annona squamosa* leaves ethanolic extract at the dose of 100mg/kg was found to suppress delayed time hypersensitivity reaction induced by SRBCs in mice. It reveals the effect of drug on T-lymphocytes and other cell types required for expression of humoral response to SRBCs.

Conclusion

The present investigation suggests that the ethanolic extract of *Annona squamosa* leaves stimulates cellular immune response that is activation of T-lymphocytes and B-lymphocytes. The effectiveness of EEAS treated animals in overcoming the side effects of drug-induced

myellosuppression provides sufficient evidences for balancing and adaptogenic efficacy.

The ethanolic extract of *Annona squamosa* leaves shown stimulant effect on non-specific arm of immune system by increasing the polymorphonuclear neutrophils margination in the blood vessels and reaching the site of inflammation and phagocytic index through activation of reticuloendothelial system in pyrogallol-induced immunosuppressed mice.

Result indicates and shown better Immunomodulatory in experimental mice models, it may be due to the presence of tannins, flavonoids and other poly phenolic compounds. Hence, the research justifies that ethanolic extract of *Annona squamosa leaves* can be effectively used in treatment of Immunomodulatory.

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Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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