Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Suppl 3, 2012

Research Article

STIMULATION OF IMMUNE SYSTEM FUNCTION BY POLYSACCHARIDES OF *MANILKARA HEXANDRA* (ROXB.) BARK

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Received: 23 Jan 2012, Revised and Accepted: 25 Mar 2012

ABSTRACT

The main aim of the present investigation was to evaluate the stimulating effect of polysaccharides from *Manilkara hexandra* bark on immune system. Firstly crude polysaccharides were extracted from *Manilkara hexandra* bark using standard procedure and the acute toxicity study was performed according to OECD guidelines. Polysaccharides at dose level of 250 and 500 mg/kg were administered seven days to the experimental animals orally and Septilin syrup was used as standard. At the end of seven days, blood was collected from retro orbital plexes and the immunomodulatory property was assessed using four methods, viz Humoral immune response, Cellular immune response, White blood cell count and Phagocytic index. The results were found that the polysaccharides from *Manilkara hexandra* bark significantly stimulating the immune system function. This activity may be due to the stimulation of macrophage function which is a known action of botanical polysaccharides.

Keywords: *Manilkara hexandra,* Polysaccharides, Humoral immune response, Cellular immune response and Phagocytic index

INTRODUCTION

Manilkara hexandra (*Mimusops hexandra)* is an evergreen tree belongs to family *Sapotaceae*. The *Manilkara* is a genus of trees in the family of *sapotaceae*. Collectively known as *Manilkara* trees, they occur throughout the tropics. Trees of this genus yield edible fruit, useful wood and latex The best-known species are *M. bidentata* (*Balata*), *M. chicle* (*Chicle*) and *M. zapota* (*Sapodilla*) [1]. The bark is astringent, sweet refrigerant, aphrodisiac, alexipharmic and anthelmintic, it is useful in uiorrhagia, ulitis, odontopathy, fever, colic dyspepsia, helminthiasis, hyper dyspepsia, burning sensation and vitiated conditions of pitta, it retards the fermentation process in toddy [2].

Macrophage activation by plant polysaccharides is thought to be mediated primarily through the recognition of polysaccharide polymers by specific receptors. Treatment of macrophages with plant polysaccharides has also been reported to modulate expression of various cell surface receptors, including those which recognize plant polysaccharides [3,4]. There are a number of plant derived polysaccharides were studied for their immunomodulatory property, a few of them includes *Aloe vera*, *Crocus sativus* , *Morinda citrifolia*, *Panax ginseng*, *Pinus parviflora*, *Trigonella foenum-graecum.* This kind of scientific study has not been documented so far, for the plant *M. hexandra*, which is a ever green forest tree and various parts are used in the treatment of few disorders. Most of the literature available on this plant was based on traditional or folklore information. Few reports are available on the actions of this plant for its effects on ulcers [5,6], antimicrobial activity [7], antibacterial activity [8]. So, we planned that it is worthwhile to carry out the immunomodulatory activity of polysaccharides of *Manilkara hexandra.* Hence in the present work an attempt was made to isolate and screen immunomodulatory property of polysaccharides in the bark of *Manilkara hexandra* in a scientific way.

MATERIALS AND METHODS

Plant material

Manilkara hexandra bark was collected from Kakatiya University medicinal garden, Warangal district, Andhra Pradesh, India and taxonomically identified and authenticated by the Dr. Raju S. Vastvya, Professor, Department of Botany, Kakatiya University, Hanamkonda. A. P., India. A voucher specimen (PG/2011/01) was deposited in department of Pharmacognosy and Phytochemistry, Vaagdevi college of pharmacy, Hanamkonda, A.P., India for future reference. The collected bark was shade dried; powdered using a mechanical grinder and powder was used for the extraction of the polysaccharides.

Animals

Wistar strain of Male Albino Rats aged about 7-8 weeks, approximately weighing between 150-200gm and Male Swiss Albino mice aged about 4 weeks, approximately weighing between 25-30gm were used in the present study. All the study protocols were approved by Institutional Ethical Committee of Vaagdevi College of pharmacy, Hanamkonda; vide approval number CPCSEA/VCOP/2011/10/3/15.

Extraction of polysaccharides

960 g of the *Manilkara hexandra* bark powder was allowed to stand in 1 L of 0.1 N Hydrochloric acid for overnight at room temperature. The extract was filtered through a typical woman's nylon cloth. Then the filtrate was neutralized with 1 N sodium hydroxide, and polysaccharides were precipitated with 3 volumes of ethanol. After centrifugation for 30 min 4000 rpm, the precipitate was re-dissolved in distilled water. Then the pH of the suspension was adjusted to 2.0 with 1 N Hydrochloric acid and Calcium chloride was added to the final concentration of 2 M. The resulting precipitate was removed by centrifugation and the supernatant was treated with 3 volumes of ethanol. The ethanol precipitation was repeated twice and the precipitate was re-dissolved in distilled water, and evaporated to get crude polysaccharides designated as MHPS [9].

Acute toxicity studies

Acute oral toxicity studies are performed as per OECD-423 guidelines (acute toxic class method). Male Swiss Albino mice were selected randomly and divided into two groups (n=3). The animals fasted over night and MHPS at the highest dose of 1000 mg/kg b.w administered orally to one group of animals. Another group received vehicle (Normal Saline) served as control. The animals were observed continuously for 24 hr, and then intermittently. Any behavioral changes / mortality were observed.

Immunomodulatory activity

Antigenic material

The sheep red blood cells (SRBCs) were used as an antigenic material. The sheep blood was obtained from slaughter house collected in Alsever's solution. During the experimentation, adequate amount of SRBCs were washed 3 times with pyrogen free control saline (0.9% w/v Nacl). The settled SRBCs were found to be 4.8×10^6 cells/mm^3 (by Haemocytometer) and used for immunization and challenge.

In vivo Humoral immune response, Cellular immune response and WBC count

Experimental rats were randomly divided into four groups and each group consists of six animals (n=6). Animals from all the groups were kept fasting over night before the day of starting the experiment. The animals were immunized by injecting 50 µl of

SRBCs suspension containing 4.8 × 10^6 cells/ mm^3 intra peritonially on day 0. MHPS of different concentrations i.e., 250, 500 mg/kg and standard Septilin syrup at dose of 1ml/100gm were administered to the respective groups orally for 7 days where as control group received normal saline. Blood samples were collected in micro centrifuge tubes from individual animal by retro orbital puncture on day 8. The blood samples were centrifuged and serum was obtained.

Humoral immune response

Antibody levels were determined by the haemagglutination technique. Briefly equal volumes of individual serum samples of each group were pooled. To serial two fold dilutions of pooled serum samples made in 25 µl volume of control saline, in U-bottomed micro titration plates were added 25 µl of freshly prepared 1% suspension of SRBCs in saline. After mixing, the plates were incubated at 37 ºC for 2 hrs and examined visually for agglutination. The highest dilution of test serum causing maximum visible haemagglutination was taken as the antibody titer [10-12].

Cellular immune response

After blood collection on day eight the thickness (ml) of the right hind foot pad was measured using plethysmometer. The rats were then challenged by injection of 25µl of 4.8 × 10^6 cells/mm^3 SRBCs subcutaneously into right hind foot pad. Foot thickness was measured again after 24 hrs after this challenge. The difference between the pre and post challenge foot thickness was taken as a measure of DTH [10,12].

White blood cell count

The number of White blood cells in the blood collected from animals in all the groups was counted using Neubaur's chamber[11].

Carbon clearance test

Adult male Swiss mice divided into four groups consisting of six animals each. The mice were deprived of food for 24hours with free access to water. After 24 hours, Septilin syrup (1ml/100gm), and extracted polysaccharides with 250mg/kg and 500 mg/kg dose was selected for screening and they were administered orally for 7 days. On day 8, the mice were injected with 0.1 ml of carbon ink (Camel fountain pen ink) suspension (1.6% v/v in 1% Gelatin, in saline) via the tail vein. Blood samples (about 50 µl) were drawn (in 0.15% w/v disodium EDTA solution, 50 µl) from the retro orbital vein, immediately (0 min) & 15 minutes after injection. A 25 µl sample was mixed with 0.1% sodium carbonate solution (2 ml) and the absorbance was measured at 660 nm taking 0.1% sodium carbonate solution as blank [12].

The carbon clearance was calculated using the following equation:

$$\text{Carbon clearance} = \frac{\text{Log OD}_1 - \text{Log OD}_2}{T_2 - T_1}$$

Where, OD1, OD2 are the optical densities at t_1 and t_2 respectively.

t_1 ---- 0 min

t_2 ---- 15 min

Statistical Analysis

All the data was expressed as Mean ± S.D. Statistical significance between more than two groups was tested using one way ANOVA followed by the Dennett's test using computer based fitting program (Prism graph pad version 5.0). Statistical significance was set accordingly.

RESULTS

Extraction

Extraction of polysaccharides from *Manilkara hexandra* were carried out using chemical method as described earlier and 16.66 gm of crude polysaccharides was obtained and the percentage yield was found to be 1.73% w/w.

Acute toxicity studies

Manilkara hexandra polysaccharides was found to be safe since no animal died even at the single dose of 1000 mg/kg when administered orally, and the animals did not show any gross behavioural changes. Hence, 1000mg/kg was considered as the safe dose.

Immunomodulatory activity

Immunomodulatory property of polysaccharide fraction of *Manilkara hexandra* (MHPS) was screened by using four methods named as Humoral immune response, WBC count, cellular immune response, and Carbon clearance test.

In humoral immune response, the polysaccharide fraction showed the haemagglutination titre (dilution) at 64 times for 250mg/kg and 256 times for 500mg/kg b.w. and standard that is Septilin syrup at 512 times which were comparable with the Control group value that is 4 times. The effect of MHPS on humoral immune response was depicted in table 1.

Table 1: Effect of Polysaccharide fraction of *Manilkara hexandra* bark on Antibody titer, WBC count, cellular immune response and Phagocytic response

S. No.	Group	HA Titer (Dilution)	WBC Count (X1000/mm^3)	DTH Response (Paw edema) ml	Phagocytic Response
1.	Control	4 times	4.13+0.14	0.105+0.008	0.010+0.004
2.	MHPS (250mg/kg)	64 times	6.19+0.28***	0.207+0.041**	0.015+0.003*
3.	MHPS (500mg/kg)	256times	9.22+0.67***	0.296+0.052***	0.02+0.002***
4.	Septilin Syrup (1ml/ 100gm)	512 times	10.83+0.78***	0.4+0.070***	0.03+0.06***

All values are shown as Mean ± SD and n=6.

*P < 0.05 – Statistically significant; **P < 0.01 – Statistically very significant; ***P < 0.001 – Statistically very highly significant in response to Control.

In WBC count, the polysaccharide fraction increased significantly (p<0.001) the WBC count to 6190 cells/cmm at 250mg/kg and 9220 cells/cmm at 500mg/kg dose and 1083 cells/cmm in standard group (p<0.001) which was 4130 cells/cmm in Control group animals. The effect of MHPS on WBC count was depicted in table 1.

In cellular immune response, the polysaccharide fraction showed a marked increase in the paw volume which was monitored by plethysmometer. It showed 0.207 ml and 0.296 ml increase in paw volume at dose levels of 250mg (p<0.01) and 500mg/kg b.w. (p<0.001) respectively which was comparable with the Control group 0.11ml and standard group which showed 0.4 ml (p<0.001). The effect of MHPS on cellular immune response was depicted in table 1.

In carbon clearance test, polysaccharide fraction showed a recognizable Phagocytic index of 0.0155 and 0.020 at dose levels of 250mg and 500mg/kg b.w. (p<0.001), respectively and standard group showed at 0.03 (p<0.001), which were comparable with the Control group value that is 0.010. The effect of MHPS on carbon clearance test was depicted in table 1.

DISCUSSION

MHPS showed a eight fold increment in the humoral immune response at 500 mg/kg dose when compared with the control group which shows that MHPS augment the humoral immune response which may be by the stimulation of macrophages and B lymphocytes

subsets in the anti body production [12]. This result of delayed type hypersensitivity indicates that the extracted polysaccharides are capable of stimulating the body immune system so that the host defence mechanism will be activated.

MHPS also increased the rate of carbon clearance significantly. Phagocytosis is a process by which certain body cells, collectively known as phagocytes, ingest and removes microorganisms, effector malignant cells, inorganic particles and tissue debris. As extracted polysaccharides are showing the good carbon clearance, the above results are indicating that the extracted polysaccharides are good immunostimulant.

WBC count was also enhanced satisfactorily by MHPS which denotes that the extracted polysaccharides are exerting potent immunostimulant property. In cellular immune response MHPS showed a marked increase in the paw volume which was monitored by plethysmometer. This result of delayed type hypersensitivity indicates that the extracted polysaccharides are capable of stimulating the body immune system so that the host defense mechanism will be activated [10].

CONCLUSION

Administration of polysaccharide fraction of *Manilkara hexandra* produced a significant stimulation of immune system. So from the above results it can be concluded that the immunostimulatory property of Polysaccharides of *Manilkara hexandra* was dose dependent. However, comprehensive Phytochemical and pharmacological research should be done to find out the exact mechanism by which this extracted polysaccharides are showing potent immunostimulatory activity.

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