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Immunomodulatory Efficacy of *Terminalia arjuna* Against A*spicularis tetraptera* in Mice

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Abstract: The present investigation deals with evaluation of the immunomodulatory efficacy of *Terminalia arjuna* bark extract in *Aspiculuris tetraptera* infected mice. The plant extract were administrated to the infected mice on 18th, 19th and 20th post infection days. The immunomodulatory efficacy due to plant extract was observed on ITH, DTH and Lymphocyte response. PCA, DTH and Lymphocyte response reactions were found to be directly proportional to the dose of drug. PCA response was maximum (8.2 mm) in the group $ITTAM_5$ and minimum (5.8 mm) in the group $ITTAA_1$. DTH response was maximum (7.8 mm) in the group $ITTAM_5$ and minimum (4.9 mm) in the group $ITTAA_1$.Lymphocyte count was observed maximum (79%) in the group $ITTAM_5$ and minimum (68.6%) in the group $ITTAA_1$. Significant increase in PCA, DTH and lymphocyte responses in the infected and treated mice indicates stimulated cell mediated as well as humoral immunity. Obtained results indicate that studied plant extract can be good immunnomodulatory agent and may boost the immune response of the host but. We conclude that methanol extract of *Terminalia arjuna* is more effective than aqueous extract of *Terminalia arjuna* plant.

Key words: *Terminalia arjuna* · *Aspiculuris tetraptera* · Immunomodulation · ITH · DTH And Lymphocyte Response

INTRODUCTION

The oxyurids (Pinworm) *Aspicularis tetraptera* is common parasite of laboratory rodents. The innocuous reputation of pinworms in rodents is at least questionable as they have thought to affect weight gain and growth rate, in addition various disorders of the intestine have been attributed to pinworm [1, 2]. Parasitism and gastrointestinal nematode parasitism in particular, is arguably the most serious constraint affecting small ruminant production worldwide. Economic losses are caused by decreased production, cost of prevention, cost of treatment and the death of infected animals [3-8].

Helminthiasis is one of the most important animal diseases worldwide, inflicting heavy production losses in grazing animals. The disease is especially prevalent in developing countries in association with poor management practices and inadequate control measures. An integrated approach is required for the effective control of helminthes which includes strategic and tactical use of anthelmintics and careful management of grazing lands, including control of stocking rates and appropriate rotation strategies [9]. However, problems have emerged with the use of anthelmintics, notably the development of resistance in helminthes [10]. In addition, recognition of the antigenic complexity of parasites has slowed vaccine development. For these various reasons, interest in the screening of medicinal plants for their anthelmintic activity remains of great scientific interest despite extensive use of synthetic chemicals in modern clinical practices all over the world.

Medicinal plants used in Indian traditional medicine called Rasaynas (devoted to enhancement of body's resistance) have attracted the attention of many scientists. Most of the investigations however are carried independently without any significant interdisciplinary approach. In literature many plants have been listed having immunomodulatory effect and some of them have been proved by using modern scientific methodologies. These plants include *Allium sativum* (Lasun), *Aloe vera*

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(Gharitakumari), *Andrographispaniculata* (Kirayat), *Asparagus racemose* (Satawar), *Azadirachtaindica* (Neem), *Curcuma longa* (Haldi), *Nyctanthus arbor-tristis* (Harsinghar), *Ocimum sanctum* (Tulsi), *Panax ginseng* (Ginseng), *Phyllanthusemblica* (amla), Picror*rhizakurroa* (Kutali), *Tinosporacordifolia* (Giloe) and *Withaniasomnifera* (Ashwagandha). A range of plant products have been found to have immunomodulatory and anthelmintic properties [11].

The plant Kingdome is known to provide a rich source of botanical immunomodulator, anthelmintics, antibacterial and insecticides [12-14]. A number of medicinal plants have been used to treat parasitic infections in man and animals [15-17]. In this paper, studies on immunomodulation evaluation of *Terminalia arjuna* plants indigenous to India have been reviewed.

MATERIAL AND METHODS

Plant Extract and Chemotherapy: *Termenilia arjuna* plant (Bark) aqueous and methanol extracts were used for treatment of the induced infection. 1 ml Aqueous and methanol extract of different concentration was given to each mouse. The plant extract treatment was given on 17, 18 and 19th days post infection. Each dose was given once a day for three consecutive days (OD x 3). The different doses of the proposed plant extract were administered to the infected mice to assess their therapeutic efficacy in experimental *Aspiculuris tetraptera* infected mice.

Preparation of the Plant Aqueous Extract: For this 0.1, 0.08, 0.06, 0.04 and 0.02 g of plant part (Bark) was taken, dried and then finely ground and 10 ml of distilled water was added respectively. It was then heated until it nearly half i.e. approximately 5 ml of each concentration solution. It than was filtered by Whatman filter paper no.1. Than it was centrifuged at 2000 rpm for 10 min. the supernatant which contain clear, fresh extract of respective part of medicinal plant was used for experimental work.

Preparation of the Plant Methanol Extract: For this 0.1, 0.08, 0.06, 0.04 and 0.02 g of plant part (Bark) was taken, dried and then finely ground and 10 ml of methanol was added respectively. It was then filter with the help of Whatman filter paper no.1.Filter solution was centrifuge at 5000 rpm for 10 minutes. Pellet was discarded and supernatant was used for experimental work.

Doses of Plant Extract and Experimental Animal Groups: The following doses and concentrations of plant extract and groups of experimental animal were used.

Groups name	Doses and concentrations of plant extract
$NINTC_1$	Non infected Non treated control-1
$INTC_2$	Infected Non treated control-2.
$ITTAA_1$	Infected treated with 1 ml of 0.02gm/10mlconcentration of *Terminaliaarjuna*aqueous extract.
$ITTAA_2$	Infected treated with 1 ml of 0.04gm/10mlconcentration of *Terminaliaarjuna*aqueous extract.
$ITTAA_3$	Infected treated with 1 ml of 0.06gm/10mlconcentration of *Terminaliaarjuna*aqueous extract.
$ITTAA_4$	Infected treated with 1 ml of 0.08gm/10mlconcentration of *Terminaliaarjuna*aqueous extract.
$ITTAA_5$	Infected treated with 1 ml of 0.1gm/10mlconcentration of *Terminaliaarjuna*aqueous extract.
$ITTAM_1$	Infected treated with 1 ml of 0.02gm/10mlconcentration of *Terminaliaarjuna*methanol extract.
$ITTAM_2$	Infected treated with 1 ml of 0.04gm/10mlconcentration of *Terminaliaarjuna*methanol extract.
$ITTAA_3$	Infected treated with 1 ml of 0.06gm/10mlconcentration of *Terminaliaarjuna*methanol extract.
$ITTAM_4$	Infected treated with 1 ml of 0.08gm/10mlconcentration of *Terminaliaarjuna*methanol extract.
$ITTAM_5$	Infected treated with 1 ml of 0.1gm/10mlconcentration of *Terminaliaarjuna*methanol extract.

Preparation of Inoculums for Infection: The dose of 100 eggs were prepared in 0.2 ml suspension of distil water and given orally with a suitable syringe fitted with a feeding needle. After inoculation, mice were kept in cages, labeled according to the experimental design, were fed routinely with the same standard diet.

Experimental Animal: Total 35 inbred female Swiss albino mice, *Musmusculus albinus* of 6-8 weeks old and 15-20 g in weight were selected. Five mice were used for positive control, 5 mice used for negative control and 25 mice used for experiment.

Experimental Parasite: For the present investigation *A. tetraptera* was selected as an experimental parasite and it being routinely maintained in the laboratory by serial passage.

Sampling: Blood from experimental and control mice was collected by cardiac puncture under mild ether anesthesia, before incision each mouse was swabbed with 90% alcohol, heart exposed, blood collected from the ventricle by a 2 ml sterilized dry glass syringe fitted with a suitable in cold overnight for clotting after which serum carefully pipetted out into clean sterilized serum collecting tubes and stored at -20°C until required.

PCA and DTH Response: The estimation of passive cutaneous anaphylaxis (PCA) was done by Ovary [18] and delayed hypersensitivity (DTH) was done by Talwar [19].

Lymphocytes Counts: This was done according to usual method of Rajgopal and Ramkrishna [20].

RESULTS

The immunomodulatory efficacy was assessed on the basis of PCA, DTH and lymphocyte response in experimental group treated with different doges of aqueous and methanolic extract of *Terminalia arjuna*. Results of PCA, DTH and lymphocyte response in mice infected and treated with different doses of drug are summarized in Tables 1 and 2 and presented by Figures 1, 2, 3 and 4.

PCA Reaction: PCA reactions were found to be directly proportional to the doses of drugs. In $INTC_2$ PCA reaction was 5.2 mm. Infected mice were treated with different doges of aqueous extract of *Terminalia arjuna* bark, PCA response were found to be 5.8, 6.1, 6.8, 7.3 and 7.6 mm at 1 ml dosage of different concentrations 0.02, 0.04, 0.06 0.08 and 0.1g/10 ml respectively. Infected mice were treated with different doges of methanol extract of *Terminalia arjuna* bark, PCA response were found to be 6.2, 6.9, 7.4, 7.9 and 8.2 mm at 1 ml doge of different concentrations 0.02, 0.04, 0.06, 0.08 and 0.1g/10ml respectively. PCA reaction was observed maximum (8.2 mm) in the group $ITTAM_5$ and minimum (5.8 mm) in the group $ITTAA_1$.

DTH Reaction: DTH reactions were found to be directly proportional to the doses of drugs. In $INTC_2$ PCA reaction was 4.5 mm. Infected mice were treated with different dosage of aqueous extract of *Terminalia arjuna* bark, DTH response were found to be 4.9, 5.2, 5.8, 6.6 and 7.2 mm at 1 ml doge of different concentrations 0.02, 0.04, 0.06, 0.08 and 0.1g/10ml respectively. Infected mice were

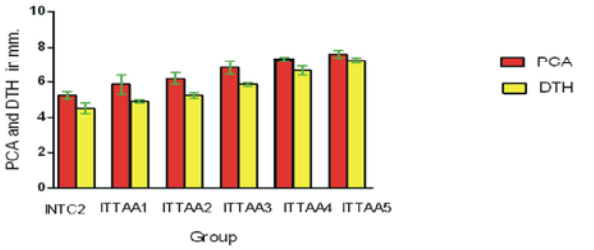

Fig. 1: PCA and DTH response from *A. tetaptera* infected mice treated mice treated with different concentration of aqueous extract of *Terminalia arjuna* (Bark)

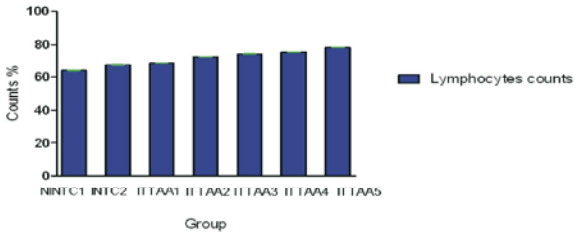

Fig. 2: Lymphocyte response from *A. tetraptera* infected mice treated with different concentration of aquwous extract of *Terminalia arjuna* (Bark)

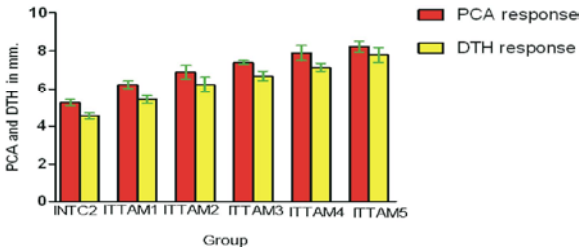

Fig. 3: PCA and DTH response from *A. tetaptera* infected mice treated mice treated with different concentration of methanol extract of *Terminalia arjuna* (Bark)

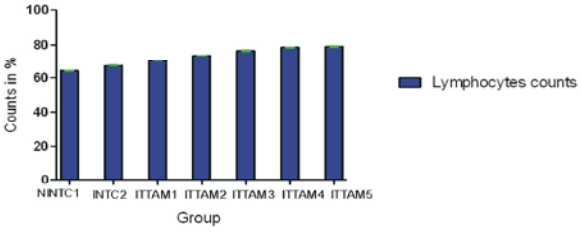

Fig. 4: Lymphocyte response from *A. tetraptera* infected mice treated with different concentration of methanol extract of *Terminalia arjuna* (Bark)

treated with different doses of methanol extract of *Terminalia arjuna* bark, DTH response was found to be 5.4, 6.2, 6.7,7.1 and 7.8 mm at 1 ml doses of different concentrations 0.02, 0.04, 0.06, 0.08 and 0.1g/10ml

Table 1: PCA, DTH and lymphocyte response from *A. tetraptera* infected mice treated with different concentrations of aqueous extract of *Terminalia arjuna* (Bark)

Group No.	Group Name	Dose and concentration	PCA response in mm. ± S.D.	DTH response in mm. ± S.D.	Lymphocyte counts in %. ± S.D.
1.	$NINTC_1$	-	-	-	64.2± 0.200
2.	$INTC_2$	-	5.2 ± 0.208	4.5 ±0.300	67.4± 0.306
3.	$ITTAA_1$	1ml of 0.02gm/10ml	5.8± 0.551	4.9± 0.100	68.6± 0.115
4.	$ITTAA_2$	1ml of 0.04gm/10ml	6.1± 0.265	5.2± 0.153	72.4± 0.153
5.	$ITTAA_3$	1ml of 0.06gm/10ml	6.8± 0.400	5.8± 0.115	74.2± 0.200
6.	$ITTAA_4$	1ml of 0.08gm/10ml	7.3± 0.100	6.6± 0.252	75.3± 0.153
7.	$ITTAA_5$	1ml of 0.1gm/10ml	7.6± 0.200	7.2± 0.153	78.1± 0.115

Skin reactions greater than 5 mm in diameter are considered significant.

Table 2: PCA, DTH and lymphocyte response from *A. tetraptera* infected mice treated with different concentrations of methanol extract of *Terminalia arjuna* (Bark)

Group No.	Group Name	Dose and concentration	PCA response in mm. ± S.D.	DTH response in mm. ± S.D.	Lymphocyte counts in %. ± S.D.
1.	$NINTC_1$	-	-	-	64.2± 0.153
2.	$INTC_2$	-	5.2 ±0.153	4.5 ±0.153	67.4± 0.300
3	$ITTAM_1$	1ml of 0.02gm/10ml	6.2± 0.208	5.4± 0.200	70.4± 0.173
4.	$ITTAM_2$	1ml of 0.04gm/10ml	6.9± 0.361	6.2± 0.404	73.5± 0.153
5.	$ITTAM_3$	1ml of 0.06gm/10ml	7.4± 0.100	6.7± 0.265	76.8± 0.360
6.	$ITTAM_4$	1ml of 0.08gm/10ml	7.9± 0.400	7.1± 0.208	78.5± 0.100
7.	$ITTAM_5$	1ml of 0.1gm/10ml	8.2± 0.289	7.8± 0.400	79.0± 0.436

Skin reactions greater than 5 mm in diameter are considered significant.

respectively. DTH reaction was observed maximum (7.8 mm) in the group $ITTAM_5$ and minimum (4.9 mm) in the group $ITTAA_1$.

Lymphocyte Counts: Lymphocyte counts were found to be directly proportional to the doses of drugs. The lymphocyte in NINTC1 group was 64.2%. In $INTC_2$ its value reached to 67.4%. Infected mice were treated with different doses of aqueous extract of *Terminalia arjuna* bark, lymphocyte counts were found to be 68.6, 72.4, 74.2, 75.3 and 78.1% at 1 ml doge of different concentrations 0.02, 0.04, 0.06, 0.08 and 0.1g/10ml respectively. Infected mice were treated with different doges of methanol extract of *Terminalia arjuna* bark, lymphocyte counts were found to be 70.4, 73.5, 76.8, 78.5 and 79% at 1 ml doge of different concentrations 0.02, 0.04, 0.06, 0.08 and 0.1g/10ml respectively. Lymphocyte counts was observed maximum (79(%) in the group $ITTAM_5$ and minimum (68.6) in the group $ITTAA_1$. All the values obtained in the various experimental groups were statistically found significant. From the above mentioned results, we conclude that methanol extract of *Terminalia arjuna* is more effective than aqueous extract of *Terminalia arjuna* plant.

DISCUSSION

The pinworm, *Syphacia muris, Syphacia obvelata* and *Aspicularis tetraptera,* are common parasites of laboratory rodents. The innocuous reputation of pinworms in rodents is at least questionable as they have been thought to affect weight gain and growth rate, in addition various disorders of the intestine have been attributed to pinworm [21-26]. Despite the wide prevalence of pinworm infection, its chemotherapy has remained surprisingly backward during the past decade. No dependable and effective drug has yet been discovered against the pinworm. Majority of the available anticestode agents do not fulfill the requirement of an ideal drug.

In the present study PCA reactions were found to be directly proportional to the doses of drug. In $INTC_2$ PCA reaction was 5.2 mm. In the experimental groups the PCA reaction was observed maximum (8.2 mm) in the group $ITTAM_5$ and minimum (5.8 mm) in the group $ITTAA_1$. PCA reaction increased in all experiments groups as compare to control group. Increase in PCA reactions after chemotherapy indicates the stimulation of reaginic (IgE) response by the test drugs as these are the only type of antibodies which are involved in anaphylactic reactions, role of reaginic antibodies in killing/expulsion of helminthic parasites conferring protection to the host is well known [27-31] and their increased levels after specific chemotherapy clearly indicate the synergism between drug activity and reaginic response. In the Present study results on PCA reaction support the finding of previous results.

In the present study DTH reactions were found to be directly proportional to the doses of drug. In $INTC_2$ PCA reaction was 4.5 mm. In the experimental groups the DTH

reaction was observed maximum (7.8 mm) in the group $ITTAM_5$ and minimum (4.9 mm) in the group $ITTAA_1$. DTH reaction increased in all experiments groups as compare to control group.

The present experiments confirm previous findings that antihelminthic agent that can increase delayed type hypersensitivity and/or T-cell mediated immunity [29-35]. DTH reactions for infected mice treated with selected natural plant products against allergen induced inflammation as suggested by [36-37]. Present study also stated that studied herbal drug results in DTH reaction were significant in all the cases.Increase in PCA as well as DTH values indicate that in *A. tetraptera* infection both the humoral and cell-mediated immunity play vital roles and thus increase markedly after drug treatment.

Lymphocyte counts were found to be directly proportional to the doses of drug. The lymphocyte in NINTC1 group was 64.2%. In $INTC_2$ its value reached to 67.4%. In the experimental groups the lymphocyte counts was observed maximum (79(%) in the group $ITTAM_5$ and minimum (68.6) in the group $ITTAA_1$. The increase in the count of lymphocyte in the drug treated groups indicated the enhancement of immunity by test drugs. Lymphocytes are the principal cells which play main role in the induction of immunity. The increase in the number of lymphocytes in the infected groups confirms similar observations by previous authors [36-37] and directs towards the involvement of lymphocyte mediated cellular immunity in helminthic infection.

*T. arjuna*is found throughout the semitropical and tropical parts of India. This is used as medicinal plant in Ayurveda and Siddha systems of medicine. It has anti-inflammatory, analgesic and immunostimulatory properties. [38]. Many authors were reported ayurvedic drugs play the main role in the increasing of WBC during infection [39-40]. The herbal immuno modulator containing *O. sanctum, Philanthus emblica,Withania somnifera*, *T. arjuna*and *Shilajit* is very helpful in boosting the immune system and fighting against *Caecal coccidosis* [11]. Lymphocyte proliferation of *O. basilicum, P. Americana, P. virginica and Rosa spp.*, were studied by Gomez-Floores [40]. He concluded that methanol and aqueous extract of *O. basilicum* showed 80 and 83% of lymphocyte proliferation, respectively. It may be due to presence of flavonoids and terpenoids. Mediratta [41] reported that *O. basilicum* modulate both humoral and cell-mediated immune responses.

The immunomodulatory effect of herbal drugs was studied by many scientists [11, 42-43]. All above authors were reported herbal drugs are very helpful in boosting the immune system and fighting against infection. In the present investigation studied herbal drugs were found to be capable for boosting of immune system against parasitic infection. Thus the result of present study supported by above mentioned authors.

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