SYNERGISTIC IMMUNOSTIMULATORY ACTIVITY OF *Terminalia bellerica* GUM POLYSACCHARIDE WITH LEVAMISOLE

Das Biswajit¹*, Dash Suvakanta¹, Choudhury Ramesh Chandra², Chakraborty Jashobir¹

¹Girijananda Chowdhury Institute of Pharmaceutical Science, Guwahati 781017, Assam, India.

²Department of Zoology, Berhampur University, Berahampur 760007, Odisha, India.

ABSTRACT

The present investigation deals with the immunosynergistic activity of *Terminalia bellerica* gum polysaccharide (TBGP) in combination with drug levamisole on experimental model. Cellular immunity was carried out by neutrophil adhesion test and carbon clearance assay, whereas, humoral immunity was analyzed by Haemagglutination antibody (HA) titer and SRBC-Induced Delayed-type hypersensitivity (DTH) response. Individual dose of TBGP was selected by Stair case method (up and down) and administered at 100, 500 & 1000 mg/kg orally and in combination with levamisole-50 respectively. The established “EXTRAIMMUNE” tablet was used as positive control and immunosuppressant Cyclophosphamide (100mg/kg/day, p.o) used as negative control. TBGP at 500 and 1000 mg/kg produced significant increases in adhesion of neutrophils and an increase in phagocytic index in carbon clearance assay whereas combination of both shows more significant result. In case of the Effect of TBGP and TBGP in combination of Levamisole on humoral response influence on sheep erythrocyte specific HA titer in mice, cyclophosphamide showed significant inhibition in antibody titer response, while TBGP and specially TBGP with levamisole shows enhanced humoral responses.

Keywords: *Terminalia bellerica* gum polysaccharide (TBGP), Delayed-type hypersensitivity (DTH), Cyclophosphamide.
INTRODUCTION
Immunostimulants or immunopotentiators are drugs those predominantly showing non-specific stimulation of immunological defense mechanisms. Most of them are not real antigens but antigen-mimetics or so-called mitogens causing non-specific and non-antigen dependent stimulation. In the indigenous system of medicines the use of herbs as immunomodulator, which can modulate the body’s defence mechanism has been reported. The active constituents of plant derivatives such as polysaccharides, lectins, peptides, flavonoids and tannins have been reported to modulate the immune system in different animal experimental models. Among the various bioactive components which have been demonstrated to be most effective as immunomodulatory and antitumor agents are polysaccharides and polysaccharo-peptides. Phagocytic cells, such as macrophages and neutrophil play a key role in innate immunity because of their ability to recognize, ingest, and destroy many pathogens by oxidative and non oxidative mechanisms (1-3).

During the past three decades, a number of bioactive polysaccharides and polysaccharide-protein complexes have been isolated from mushrooms, fungi, yeast, algae, lichens, and plants, and these compounds have attracted significant attention because of their Immunomodulatory and antitumor effects. Polysaccharides have been shown to enhance the host’s immune response by stimulating the production of macrophages, NK cells, and T-lymphocytes. Numerous studies have shown that polysaccharides produce their anti-cancer effect by enhancing the host’s immune system rather than via a direct cytotoxic effect by the stimulation of macrophages, natural killer (NK) cells, and cytotoxic T-lymphocytes (CTL) activities along with their secretory products like TNF, reactive nitrogen and oxygen intermediates, and interleukins (4, 5).

Various parts of the plant of **Terminalia bellerica** used for the treatment of a variety of disorders. Form literature it was found that extract from fruit of **Terminalia bellerica** stimulates immune system by acting through cellular and humoral immunity in experimental models of immunity in animals (6, 8).

In recent time various bioactive polysaccharide were reported to combine with other drugs showing promising synergistic effect in the treatment of various diseases. For example- PSK (Protein bound β-glucan) has been developed in Japan as a non-specific immunostimulant and has been used for the treatment of gastric and colorectal cancers. A ginseng
polysaccharide injection has been developed in China as a useful adjuvant for irradiation therapy and chemotherapy for cancer patients (9-12).

Levamisole acts as an anti-parasitic, immunomodulator and adjuvant in colorectal cancer. It appears to restore depressed immune function through stimulating antibody formation and enhance T-cell response by stimulating T-cell activation and proliferation. From the literature of chapter 2 it was found that Immunostimulatory plant like Asparagus racemosus, Sida cordifolia in combination with levamisole produce significant immunostimulation in bird. However, information about effect of purified polysaccharide and Levamisole in relation to immunological change in experimental animal is not adequately available till date (13-15).

In the present study immune synergistic activity of levamisole along with polysaccharide extracted from Terminalia bellerica gum has been studied

MATERIALS AND METHODS

Materials
Levamisole was kindly gifted by Wockhardt Limited, Baddi, India. Extra immune tablets (Charaka pharmaceuticals) and Cyclophosphamide in the form of Cycloxan-50 tablet were locally purchased from the medicine store. Nylon fibre purchased from local market Guwahati, Assam, India.

Plant material
Purified polysaccharide of Terminalia bellerica Gum (isolation and purification procedure mentioned in chapter- 4) used as test material along with drug along with levamisole.

Experimental animals
Laboratory bred swiss albino mice (20-25g) of either sex were housed at 25 ± 2°C with a relative humidity of 30–70% and illumination cycle set to 12 h light and 12 h dark. The animals were free access to standard food pellets containing (% w/w) protein 22.10, oil 4.13, fibre 3.15, ash 5.1, silica 1.12 and water ad libitum. Bedding material was removed and replaced with fresh peddy husk as often as necessary to keep the animal clean and dry. The animals were maintained under standard conditions in animal house approved by committee for the purpose of control and supervision on experiments on animals (CPCSEA). The experimental protocol was approved by the Animal Ethics Committee of Girijananda
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Choudhury Institute of Pharmaceutical Sciences, Guwahati, Assam (16). (CPCSEA Reg. No.1372/C/10/2013 CPCSEA, Study approval No-GIPS/IAEC/10/2013)

Selection of Doses form

Terminalia bellerica Gum polysaccharide (TBGP) were safe at limit dose of 2000 mg/kg, in studied subjects as per acute toxicity study result (Chapter-5). 1/20<sup>th</sup>, 1/4<sup>th</sup> and 1/2 of this dose i.e. 100 mg/kg, 500 mg/kg and 1000mg/kg for TBGP were used in the subsequent study and in combination with 50mg/kg of Levamisole respectively. Oral suspensions of the TBGP and Pure Levamisole Hydrochloride compounds were prepared by suspending them separately in 1% solution of sodium carboxyl-methylcellulose to obtain suitable dosage forms (6, 17).

Preparation of Sheep red blood cells (SRBCs)

Fresh, blood of a healthy sheep were collected from the local slaughterhouse and kept in alsever’s solution. Red blood cells from sheep blood (SRBCs) were than isolated to act as antigenic material. Adequate amount of SRBCs were washed minimum 3 times with pyrogen free control saline (0.9% w/v Nacl) during the experimentation. SRBCs concentration adjusted to 1× 10<sup>8</sup> cells/mm<sup>3</sup> (by Haemocytometer) for immunization and challenge (18).

Preparation of carbon ink suspension

Carbon ink suspension was prepared from camel fountain pen ink. Ink suspension was diluted eight times with normal saline and used for carbon clearance test. A dose of 10µl/gm body weight of mice is recommended for carbon clearance test (18).

Acute toxicity study

Healthy male and female Swiss albino mice (8 weeks) were used for the acute oral toxicity study. They were breed and reared at the animal house of the institution. The animals were housed in polypropylene cages and provided with bedding of clean paddy husk. The animals were acclimatized to laboratory conditions for one week prior to the experiment. The temperature in the animal house was maintained at 25 ±2°C with a relative humidity of 30–70% and illumination cycle set to 12 h light and 12 h dark. The mice were fed with standard laboratory pelleted feed (M/s Gold Mohur Foods and Feeds Ltd. Bangalore, India). All the mice of both the sexes were fasted overnight before experimentation and were allowed to take food one hour after the experiment. Purified polysaccharide of TBG was administered orally at a dose of 2000 mg/kg body weight in distilled water. The animals were observed for any mortality and morbidity (convulsions, tremors, and grip strength and pupil dilatation) at
an interval of 12 h for 14 days. This study was approved by the Animal Ethics Committee of Girijananda chowdhury institute of pharmaceutical sciences (CPCSEA Regn.No.1372/C/10/2013/CPCSEA, study approval No-GIPS/IAEC/10)

Protocol of the study

The drug solutions were prepared with distilled water for oral administration. Immunomodulatory activities were checked both at cellular and humoral levels. Cellular immunity was evaluated by Neutrophil adhesion test and Carbon clearance assay where as; humoral immunity was analyzed by Delayed type hypersensitivity test and indirect haemagglutination assay. All the experimental models had ten (10) common groups consisting of six (6) animals each. Group-I, was treated with control vehicle (vehicle i.e. Normal saline 1 ml/100 g/day p.o). Group II animals were marked as positive control by treating them with marketed EXTRAIMMUNE tablets which is triturated and suspended in distilled water (100mg/kg/day p.o). Group III animals marked as negative control group treated with immunosuppressant Cyclophosphamide (100mg/kg/day, p.o). Group IV animals were treated with pure drug suspension of Levamisole 50mg/kg/p.o. Group V, Group VI and Group VII animals were treated with low (100 mg/kg/day p.o) medium dose (500 mg/kg/day p.o) and high dose (1000 mg/kg/day p.o) of purified polysaccharide isolated from Terminalia bellerica Gum respectively. Group VIII, Group IX and group X animals were received 100mg/kg/day p.o, 500mg/kg/day p.o and 1000 mg/kg polysaccharide with levamisole (50mg/kg/day,p.o.) in combination (18, 19).

Swiss albino mice were administered with TBG Polysaccharide, vehicle, Cyclophosphamide and TBGP with levamisole orally to the respective groups of animals for 10 days as per experimental protocol. Forty eight hours after the last dose of the drug, animals of all the groups received intravenous injection of (10µl/gm) Indian colloidal carbon ink (Camel fountain pen ink) suspension via the tail vein. Blood samples were withdrawn from each mouse by retro-orbital plexus at an interval of 0 and 15 min after the ink injection. A 50-µl blood sample was mixed with 4 ml of 0.1% sodium carbonate solution and the absorbance of this solution was determined at 660 nm taking 0.1% sodium carbonate solution as blank (6, 17 and 18). The phagocytic index K was calculated using the following formula,

\[ K = \frac{\log_{e} OD1 - \log_{e} OD2}{15} \]
Where, OD1 and OD2 are the optical densities at 0 and 15 min, respectively.

**Neutrophil adhesion Test**

The experimental Swiss albino mice were pre-treated orally with vehicle and TBGP according to the proposed experimental protocol for 14 days. At the end of treatment day, blood samples were collected from the retro-orbital plexus into heparinized vials and analyzed for differential leukocyte count (DLC). After the initial counts, blood samples were incubated with 80 mg nylon fibres/ml for 15 min at 37°C. The incubated blood samples were again analyzed for TLC and DLC, respectively to give neutrophil index of blood samples \(^{(20-21)}\). The percentage of neutrophil adhesion was calculated as follows,

\[
\text{Neutrophil adhesion (\%)} = \frac{N_{lu} - N_{lt}}{N_{lu}} \times 100
\]

Where \(N_{lu}\) is the neutrophil index of untreated blood samples and \(N_{lt}\) is the neutrophil index of treated blood samples.

**In-vivo humoral antibody titer and delayed type hypersensitivity response**

**Haemagglutination antibody (HA) titer**

The animals of all the groups were immunized by injecting 0.1 ml of SRBCs suspension containing \(1\times10^8\) cells intraperitoneal on day 0. The drug treatment was continued for 14 more days. Blood samples were collected in microcentrifuge tubes from individual animal of all the groups by retro-orbital vein puncture on day 14. The blood samples were centrifuged and serum was separated. Antibody levels were determined by the haemagglutination technique. Equal volumes of (50 µl) individual serum samples of each group were pooled. To serial two fold dilutions of pooled serum samples made in 50 µl volumes of RPMI-1640 in micro titration plates, 50 µl of 1% suspension of SRBC in RPMI-1640 was added. After mixing, the plates were incubated at 37°C for 1 h and examined for haemagglutination under microscope (button formation). The reciprocal of highest dilution, just before the button formation, was observed as the titer values of the test samples. The antibody titers were expressed in the graded manner, the minimum dilution (1/2) being ranked as 1 (reciprocal dilution), and mean ranks of different groups were compared for statistical significance. The concentration of antibodies (\(A_b\)) present in blood serum expressed in µl which is required for haemagglutination also calculated \(^{(6, 22-24)}\).
SRBC-Induced Delayed-type hypersensitivity (DTH) response
Grouping and dosing of all animals were done exactly same as haemagglutination antibody titer test. The delayed type hypersensitivity test was commenced after the Humoral Antibody titer model with the same animals. After immunization of eighth day, the thickness of the right and left hind footpad were measured using Vernier calliper and volume of water displacement was measured through plethysmometer. The mice were then challenged by 1× 10⁸ SRBCs in right hind footpad 0.03 ml and normal saline in left hind paw in same volume. The footpad thickness and water displacement volume were measured again after 8, 24 and 48 h of the challenge. The difference between the pre and post challenge footpad thickness expressed in mm as well as difference in the water displacement volume in ml. Increase in the thickness of footpad as a result of hypersensitivity reaction due to edema (17, 25).

Statistical analysis
All the data were expressed as Mean ± SEM. Statistical significance between more than two groups were 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. The values were expressed as mean± SEM and P <0.001 was considered statistically very highly significant, P <0.01 considered as very significant and P<0.05 considered as significant (18).

RESULT AND DISCUSSION
Carbon clearance test
The phagocytic activity of the reticulo-endothelial system was measured by the rate of removal of carbon particles from the blood stream. The effect of TBGP and TBGP + Levamisole in different dosage forms were evaluated with respect to the phagocytic activity by the carbon clearance (Fig.1). The results in Table.1 showed increased phagocytic index of TBGP treated group significant, (*p<0.001), very significant (**p<0.001), very high significant (**p<0.001) with increase in dose (100, 500 &1000 mg/kg). When TBGP (100, 500 & 1000 mg/kg)+ Levamisole-50mg/kg combination was given to the groups (VII, IX and X respectively) it exhibited very high significant phagocytic index (**p<0.001) compared to control group. Phagocytic index of negative control group significantly decreases (*p<0.001), but in case of established marketed EXTRAIMMUNE-100mg/kg&levamisole-50mg/kg shows very significant increment of phagocytic index was seen (**p<0.001). The increased
phagocytic index reflects the enhancement of the phagocytic function of mononuclear macrophage and nonspecific immunity\(^{(18)}\).

Fig.1. Phagocytic index of Different treatment group, Where TBGP: *Terminalia bellerica* gum polysaccharide; LEV: Levamisole.

<table>
<thead>
<tr>
<th>Sl. no</th>
<th>Treatment Group</th>
<th>Phagocytic index</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Group-1(Control)</td>
<td>0.01994±0.00097</td>
</tr>
<tr>
<td>02</td>
<td>Group-II(-VE Control)</td>
<td>0.006221±0.00099*</td>
</tr>
<tr>
<td>03</td>
<td>Group-III(+VE Control)</td>
<td>0.05258±0.00495***</td>
</tr>
<tr>
<td>04</td>
<td>Group IV (Levamisole )</td>
<td>0.03168±0.00246***</td>
</tr>
<tr>
<td>05</td>
<td>Group V (TBGP100)</td>
<td>0.0354±0.00264*</td>
</tr>
<tr>
<td>06</td>
<td>Group VI(TBGP 500)</td>
<td>0.03689±0.00381**</td>
</tr>
<tr>
<td>07</td>
<td>Group VII (TBGP1000)</td>
<td>0.05154±0.00283***</td>
</tr>
<tr>
<td>08</td>
<td>Group VIII(TBGP100+Levamisole)</td>
<td>0.05171±0.002498***</td>
</tr>
<tr>
<td>09</td>
<td>Group IX(TBGP500+ Levamisole)</td>
<td>0.05482±0.00322***</td>
</tr>
<tr>
<td>10</td>
<td>Group X(TBGP1000+Levamisole)</td>
<td>0.05757±0.00206***</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± SE, n = 6 statistically significant applying the Dunnett’s test. *P < 0.001 – Statistically significant; **P < 0.001 – Statistically very significant; ***P < 0.001 – Statistically very highly significant in response to Control.

**Neutrophil adhesion test**

The neutrophil adhesion of nylon fibers describes the margination of polymorph nuclear lymphocyte in the blood vessels and the numbers of macrophages reaching the site of inflammation. Cytokines are secreted by activated immune cells for margination and extravasations of the phagocytes mainly polymorphonuclear neutrophil. Significantly evoked increase in the adhesion of neutrophil to nylon fibers which correlates to the process of
margination of cells in blood vessels. In this study effect of different treatment groups on the neutrophil activation by the neutrophil adhesion test is mentioned in Table.2. Incubation of blood with nylon fibers (NF) produced a decrease in the neutrophil counts due to adhesion of neutrophil to the fibers. Therefore, as neutrophil adhesion increases, in different treatment groups (Fig.2), the percentage of neutrophil index was found to be statistically very highly significant (**p<0.001) in treatment groups of all TBGP (100, 500 & 1000mg/kg) and TBGP (100, 500 & 1000 mg/kg) with Levamisole-50 mg/kg as compared with the Vehicle control group. Group VIII, IX and X significantly evoked increase in the adhesion of neutrophil to nylon fibers with neutrophil adhesion percentage increment 45.86±1.566%, 46.62±2.621% and 46.86±2.061% respectively. In case of negative control percentage of adhesion of neutrophil to nylon fiber non significantly decrease, but in case of established marketed immune stimulant EXTRAIMMUNE-100mg/kg treated & levamisole-50mg/kg treated group shows very highly significant increments of neutrophil adhesion (**p<0.001). From the result it was observed that when TBGP was given alone in lower or higher dose, increase in adhesion of neutrophil nylon fiber was seen in dose dependent manner (27.49±1.884, 29.12±3.058, 29.54±2.634) but not so prominent as seen in carbon clearance test. Same types of observation were found when TBGP combine with levamisole but the intensity of binding was high (26, 27).

![Graph](image-url)

**Fig.2.** Percentage of Neutrophil adhesion in different treatment group, Where TBGP: *Terminalia bellerica* gum polysaccharide; LEV: Levamisole.
Table 2. Percentage of Neutrophil adhesion in neutrophil adhesion test with their significance

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Treatment Group</th>
<th>Neutrophil adhesion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Group-I (Control)</td>
<td>8.430±1.445</td>
</tr>
<tr>
<td>02</td>
<td>Group-II (-VE Control)</td>
<td>6.567±1.758</td>
</tr>
<tr>
<td>03</td>
<td>Group-III (+VE Control)</td>
<td>48.39±0.990***</td>
</tr>
<tr>
<td>04</td>
<td>Group IV (Levamisole)</td>
<td>36.40±1.463***</td>
</tr>
<tr>
<td>05</td>
<td>Group V (TBGP100)</td>
<td>27.49±1.884***</td>
</tr>
<tr>
<td>06</td>
<td>Group VI (TBGP 500)</td>
<td>29.12±3.058***</td>
</tr>
<tr>
<td>07</td>
<td>Group VII (TBGP1000)</td>
<td>29.54±2.634***</td>
</tr>
<tr>
<td>08</td>
<td>Group VIII (TBGP100 + Levamisole)</td>
<td>45.86±1.566***</td>
</tr>
<tr>
<td>09</td>
<td>Group IX (TBGP500 + Levamisole)</td>
<td>46.62±2.621***</td>
</tr>
<tr>
<td>10</td>
<td>Group X (TBGP1000 + Levamisole)</td>
<td>46.86±2.061***</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± SE, n = 6 statistically significant applying the Dunnett’s test. *P < 0.001 – Statistically significant; **P < 0.001 – Statistically very significant; ***P < 0.001 – Statistically very highly significant in response to Control.

**In-vivo humoral antibody titer and delayed type hypersensitivity response**

**Haemagglutination antibody (HA) titer**

To evaluate the effect of TBGP and TBGP in combination of levamisole for humoral immune response, the influence was tested on sheep erythrocyte specific HA titer in mice. Cyclophosphamide-50mg/kg showed significant inhibition in antibody titer response, while TBGP in different concentration and TBGP with levamisole enhanced the humoral responses as per Fig.3 & 4. The reciprocal of humoral antibody titer value was found in the range from 7.667±1.961 to 455.3±52.18 dilution and antibody concentration required for visible clot formation is between 0.00231±0.00027 and 0.1979±0.065 µl. Administration of TBGP (100, 500, 1000 mg/kg per day for 14 days) produced a dose dependent increase of antibody against SRBC. Hence dilution factor increases in graded manner as 104.5±18.57, 120.0±16.40 and 218.3±16.82 and antibody concentration required to visible clot decreases simultaneously as 0.01077±0.00145, 0.009483±0.0014 and 0.00433±0.00059 µl (Table 7.3). At higher dose (1000 mg/kg) of TBGP and all treatment groups of TBGP with levamisole showed very highly increased significant value (***p<0.001) in the haemaglutination titer test when compared with control as per Table 3. Similiarly, the antibody concentration required for visible clot formation in negative control increased very high significantly (***p<0.001) and vice versa. All the treatment groups of TBGP with levamisole and cyclophosphamide actions exhibited remarkable differences in the humoral antibody titer (28, 30).
Fig. 3. Reciprocal of dilution factor in HA titer in different treatment group, Where TBGP: *Terminalia bellerica* gum polysaccharide; LEV: Levamisole.

Fig. 4. Concentration of antibody in µl/ml in HA titer test, Where TBGP: *Terminalia bellerica* gum polysaccharide; LEV: Levamisole.

Table 3. HA titer value in different treatment group.

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Treatment Group</th>
<th>Reciprocal dilution factor</th>
<th>Conc. of Antibody µl/ml required</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Group-I(Control)</td>
<td>30.00±4.099</td>
<td>0.03752±0.0063</td>
</tr>
<tr>
<td>02</td>
<td>Group-II(-VE Control)</td>
<td>7.667±1.961</td>
<td>0.1979±0.065***</td>
</tr>
<tr>
<td>03</td>
<td>Group-III(+VE Control)</td>
<td>336.0±22.63***</td>
<td>0.003033±0.00002</td>
</tr>
<tr>
<td>04</td>
<td>Group IV (Levamisole)</td>
<td>261.3±23.97***</td>
<td>0.003281±0.0002</td>
</tr>
<tr>
<td>05</td>
<td>Group V (TBGP100)</td>
<td>104.5±18.57</td>
<td>0.01077±0.00145</td>
</tr>
<tr>
<td>06</td>
<td>Group VI(TBGP 500)</td>
<td>120.0±16.40</td>
<td>0.009483±0.00014</td>
</tr>
<tr>
<td>07</td>
<td>Group VII (TBGP1000)</td>
<td>218.3±16.82***</td>
<td>0.00433±0.00059</td>
</tr>
<tr>
<td>08</td>
<td>Group VIII (TBGP100+Levamisole)</td>
<td>268.0±18.68***</td>
<td>0.004733±0.0007</td>
</tr>
<tr>
<td>09</td>
<td>Group IX (TBGP500+ Levamisole)</td>
<td>402.5±62.61***</td>
<td>0.002683±0.0004</td>
</tr>
<tr>
<td>10</td>
<td>Group X (TBGP1000+ Levamisole)</td>
<td>455.3±52.18***</td>
<td>0.00231±0.00027</td>
</tr>
</tbody>
</table>
All values are expressed as mean ± SE, n = 6. Statistically significant applying the Dunnett’s test. *P < 0.001 – Statistically significant; **P < 0.001 – Statistically very significant; ***P < 0.001 – Statistically very highly significant in response to Control

SRBC-Induced Delayed-type hypersensitivity (DTH) response

Delayed Type Hypersensitivity (DTH) reaction requires the specific recognition of a given antigen by activated T lymphocytes, which subsequently proliferate and release cytokines. When activated TH1 cells encounter certain antigens, viz. SRBCs. They secrete cytokines that induce a localized inflammatory reaction called delayed type hypersensitivity. The mean differences of paw edema in terms of thickness and percentage of increase in paw volume after 8 hr, 24 hr and 48 hrs were considered in hypersensitivity reaction is presented in figures.5 to 10. The result obtained in Table.4 indicated that animals treated with TBGP (100, 500, 1000 mg/kg) show little increase in paw edema (both in terms of thickness in mm and percentage of increased paw edema) in comparison with control after 8hrs of challenge with SRBC. The paw edema was significantly increased (*p<0.001) with TBGP 500, 1000mg/kg and TBGP in combination with levamisole (Group VIII, IX, X) after 8 hour, 24hr and 48 hr respectively. From DTH data it can be speculated that groups treated with TBGP at dose 100 mg/kg, 500 mg/kg, 1000 mg/kg showed increase in DTH response but not significantly, whereas TBGP of above mentioned dosage form combined with drug levamisole shows very high significant (**p<0.001) increase in DTH response(31-34).

Fig.5.Paw thickness after 8 Hr, Where TBGP: Terminalia bellerica gum polysaccharide; LEV: Levamisole.
Fig. 6. Paw thickness after 24 hr, Where TBGP: *Terminalia bellerica* gum polysaccharide; LEV: Levamisole.

Fig. 7. Paw thickness after 48 hr, Where TBGP: *Terminalia bellerica* gum polysaccharide; LEV: Levamisole.

Fig. 8. Percentage of increase foot pad oedema volume after 8 hour, Where TBGP: *Terminalia bellerica* gum polysaccharide; LEV: Levamisole.
Fig. 9. Percentage of increase of foot pad edema after 24 hours, Where TBGP: *Terminalia bellerica* gum polysaccharide; LEV: Levamisole.

Fig. 10. Percentage of increase of foot pad edema after 48 hour, Where TBGP: *Terminalia bellerica* gum polysaccharide; LEV: Levamisole.

Table 4. Changes in thickness and volume in paw edema

<table>
<thead>
<tr>
<th>Sl.no</th>
<th>Treatment Group</th>
<th>Thickness in mm</th>
<th>% increase in foot pad edema</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>8 hour</td>
<td>24 hour</td>
</tr>
<tr>
<td>01</td>
<td>Group-I (Vehicle Control)</td>
<td>0.0926 ± 0.0056</td>
<td>0.1767 ± 0.0247</td>
</tr>
<tr>
<td>02</td>
<td>Group-II (-VE Control)</td>
<td>0.0916 ± 0.0040</td>
<td>0.1367 ± 0.0210</td>
</tr>
<tr>
<td>03</td>
<td>Group-III (+VE Control)</td>
<td>0.1215 ± 0.0085</td>
<td>0.5450 ± 0.0341***</td>
</tr>
</tbody>
</table>
All values are expressed as mean ± SE, n = 6. *Statistically significant difference at p < 0.001 as compared to vehicle control as seen by applying the Dunnett’s test.

CONCLUSION

All the doses of TBGP in combination with levamisole showed remarkable augmentation in the phagocytic index due to increase in the activity of the reticulo endothelial system in carbon clearance test. Same types of activity were observed in case of neutrophil adhesion test. Haemoglutination titer value also increased in case TBGP in combination with levamisole in comparison to individual. It indicate that the combination of polysaccharide and levamisole synergistically improve the humoral antibody response. Similar types of response in DTH also indicate that the synergistic potentiality of the combination. Hence, it was concluded that polysaccharide isolated from *Terminalia bellerica* gums in combination with levamisole have synergistic immunotherapeutic potential and further studies for elucidation of the mechanism of action are recommended.

REFERENCES


