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Effect of *Boswellia serrata* Resin Extract on Cell-Mediated and Humoral Immune Response in Mice

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ABSTRACT

Globally, Boswellia serrata is used by many different communities to treat various ailments including modulation of the immune system though with limited scientific evidence. This study was aimed at investigating the immunomodulatory and antioxidant effect of Boswellia serrata resins extract in rodents. Chemical characterization of the extract was performed for detecting the presence of secondary metabolites using pure boswellic acids as standard. In vitro antioxidant assay was performed by DPPH free radical scavenging assay and reducing power assay. In vivo study includes acute toxicity assay, delayed type hypersensitivity test, Hemagglutination reaction and carbon clearance test. Enzymatic action was used for the estimation of reduced glutathione (GSH), Superoxide dismutase (SOD) and Lipid peroxidation (LPO). The sheep red blood cells (SRBCs) are used as an antigenic material. Statistical comparison between different groups was done by using one way ANOVA followed by Benferroni's test. Based on the findings from the study, it is concluded that the ethanolic extract of Boswellia serrata increased both the cell-mediated and humoral immune responses in rats. This could be attributed to the different macronutrients, micronutrients and phytochemicals present in the plant. The extract therefore has a potential therapeutic value in several immunosuppressing clinical disorders and hence the reason for its use in local communities to alleviate various disease conditions.

Keywords: Immunomodulatory, Antioxidant, Boswellia serrata, Humoral, Metabolites

INTRODUCTION

Boswellia genus contains nearly 25 distinct species and some of the important species of this genus include *Boswellia serrata, Boswellia sacra, Boswellia carterii, Boswellia papyrifera, Boswellia neglecta, Boswellia rivae, Boswellia frereana, and Boswellia ovalifoliolata*, etc. [1-4]. The gum resin of *Boswellia serrata* (BS), a traditional treatment of Ayurvedic medicine in India also identified as Indian frankincense, Salai Guggal, or Indian olibanum, has been used for centuries as a remedy for many health problems [5]. The dried gum appears in form of lumps or tears which are white-yellow in color. The word frankincense meaning "pure incense" is derived from the ancient French name. A number of review and research articles focusing on pharmacological studies have highlighted the usefulness of boswellic acids in the management of several chronic inflammatory diseases including chronic ulcerative colitis, rheumatoid arthritis, crohn's disease, and bronchial asthma; in addition to its anti-depressive and anti-anxiety effects and beneficial effects in brain tumor patients [6]. From the ancient times, the extracts from the oleo-gum resin of *Boswellia serrata Roxb*. ex Colebr. (family *Burseraceae*), are also identified as Indian frankincense or Salai Guggal, have been used in traditional Ayurvedic medicine for the treatment of inflammatory diseases including osteoarthritis and chronic bowel diseases [7-10].

The oleo-gum resin, obtained by incision of the bark, is composed by essential oil (5-9%), mucopolysaccarides (21-22%), and pure resin (65-85%), containing tetracyclic and pentacyclic triterpene acids, of which boswellic acids (BAs) are the most important bioactive molecules [11,12]. In particular, 11-keto-β-boswellic acid (KBA) and 3-O-acetyl-11-keto-β-boswellic acid (AKBA) were proposed to act as inhibitors of 5-lipoxygenase (5-LO) [13,14]. Recently, other components of the phytocomplex, for example β-boswellic acid (βBA), have been recommended as anti-inflammatory molecules, acting through inhibition of serine protease cathepsin G (catG) and microsomal prostaglandin E synthase (mPGES) [15]. The current study aimed to identify and explore the *in vivo* immunomodulatory and antioxidant effect of *Boswellia serrata*.

MATERIALS AND METHODS

Collection and preparation of test material

The plant specimens were collected from Vindhya herbals, Bhopal (M.P). *Boswellia serrata* Roxb. identified from a voucher specimen (Vindhya herbals no.- 22) in Herbarium. Extraction method is the crucial first step in the analysis of medicinal plants, because it is necessary to extract the desired chemical components from the plant materials to know its efficacy. Extraction was done by maceration. 1 kg *Boswellia serrata* Roxb gum resin was soaked in ethanol (Sigma-Aldrich) in an amber-colored bottle for 3 days with occasional shaking. The mixture was filtered on the 3rd day using a gauze cloth and the fine filtrate was obtained using Whatman No: 1 filter paper in a Buchner funnel. The filtrate was concentrated using a Rotatory evaporator which was further dried into a semi-solid extract in an oven set at 50°C. The dry extract was stored at 4°C until the immunomodulatory experimental bioassays were carried out. The percentage yield of the extract was determined which was 52 g crude extract.

Preliminary phytochemical screening

Phytochemicals screening methods for detecting the presence of secondary metabolites in *Boswellia serrata* Roxb [16].

In vitro antioxidant assay

DPPH free radical scavenging assay: Different concentrations (20, 40, 60, 80, 100 µg/mL) of test sample and 0.1mM DPPH solution was prepared in methanol. Ascorbic acid (100 µg/mL) was used as standard. 2mL of DPPH solution and 1 mL of methanol was used as control. 2 mL of plant extract of different concentrations was blended in with 2 mL of DPPH solution and control separately. The mixture was incubated for 10 minutes in the dark and absorbance was measured at 515 nm by spectrophotometer using methanol as blank. The percentage inhibition of DPPH radical was calculated by following formula [17].

$$\%Inhibition = \frac{A\ Control - A\ sample}{A\ Control} X100$$

Where Acontrol is the absorbance of the control reaction (containing all reagents except the test extract), and Asample is the absorbance of the test extract. IC50 is calculated by plotting% inhibition as a function of sample concentration. IC50 is defined as extract concentration necessary to inhibit 50% of DPPH solution.

Reducing power assay: This method is based on the principle of increase in the absorbance of the reaction mixture. Increase in the absorbance indicates increase in the antioxidant activity. Substances, which have reduction potential, react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanides (Fe^{2}+), which then react with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm. Increase in absorbance of the reaction mixture indicates the reducing power of the Samples. 1 mL of various concentrations of extract was mixed with 2.5 ml phosphate buffer solution (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The solution was properly mixed and placed in incubator for 20 min at 50ºC. After incubation, the resulting solution was cooled and 2.5 ml of 10% tri chloro acetic acid was added to reaction mixture, followed by centrifugation at 3000 rpm for 10 min. After centrifugation 2.5 ml of supernatant was mixed with equal volume of distilled water and finally 0.5 ml of 0.1% ferric chloride was added. The reaction mixture was shaken and kept at room temperature for 10 min. The absorbance was measured at 700 nm [18].

In vivo study

Animals: (*Mus musculus* L.) Swiss albino mice weighing 22 ± 5g of either sex were obtained from National Institute of Nutrition, Hyderabad and were acclimatized for 3-4 weeks in the animal house of Jawaharlal Nehru Cancer Hospital and Research Center, Idgah Hills, Bhopal. They were maintained under controlled conditions at temperature of 22 ± 2°C, humidity 60 ± 10% and a 12/12 hour light/dark cycle. They were housed in polypropylene cages containing sterile paddy husk as bedding and had free access to standard rodent pellet, and filtered and acidified water *ad libitum*. All experimental protocols were approved by Committee for the Protection and Control and Supervision of Experimental Animals (CPCSEA), prior to beginning the experiments. The Registration No. is 500/01/a/CPCSEA/2001. (PN. 670/225-IAEC /2008/Project no.49).

Acute toxicity studies: Acute toxicity studies were performed as per OECD 423 guidelines (Organisation for Economic Cooperation and Development). Swiss albino mice (18 ± 2 g) (male, n = 3), maintained at a temperature of 22°C ± 2°C, fed with standard pelleted feed and water *ad libitum* was used for the study. The animals were kept fasting for overnight provided only water. Then, the extract was administered orally by gastric intubation and observed for 14 days for toxic symptoms like behavioral change, locomotion, convulsion, and mortality.

Antigen: The sheep red blood cells (SRBCs) were used as an antigenic material. Fresh blood was collected in a mixture of 0.49% w/v EDTA and 0.9% w/v sodium chloride solution from sheep sacrificed in the local slaughter house, Bhopal, India. SRBCs were washed three times in a large volume of pyrogen free sterile normal saline by centrifugation at 3000 g for 10 min on each cycle. The washed SRBCs were adjusted to a concentration 20% v/v with normal saline. The animals were immunized by injecting 0.1 mL of 20% v/v SRBC during the treatment.

Delayed type hypersensitivity test [19]

Animals were divided into six groups of six mice each. SRBC was used as antigen in the study. In group I animals were treated with vehicle which was administered orally for five days. In group II, III and IV *Boswellia serrata* Roxb ethanolic extract was administered orally at the dose of 50 mg/kg, 100mg/kg and 150 mg/kg body weight and group V served as standard treated with Levamisole (2.5 mg/kg) respectively for five days. Extracts and vehicle was administered on each two day before immunization, on the day of immunization and on each two day after immunization (i.e. -2, - 1, 0, +1+2). Group VI received SRBC 0.1 mL of 20% v/v, i.p and serves as SRBC control. Mice were immunized by injecting 0.1 ml of SRBS subcutaneously into the right hind paw on day zero. Animals were challenged seven day later with same amount of SRBC into the left hind paw. Change in paw thickness was measured using digital caliper at 4, 24, 48 and 72 hour after challenge.

Hemagglutination reaction [20]

In the Hemagglutination reaction Group I animals were treated with vehicle orally for five days. In group II, III, IV extracts were administered orally at the dose of 50 mg/kg, 100mg/kg and 150 mg/kg body weight for five days. Extract groups (Group II, III, IV), standard-Levamisole 2.5 mg/kg (Group V) and vehicle (Group I) was administered on each two day before immunization, on the day of immunization and on each two day after immunization (i.e. -2, -1, 0, +1+2). Mice were immunized by intraperitoneal injection of 0.5 ml SRBC. Group VI received SRBC 0.1 mL of 20% v/v, i.p and serves as SRBC control. On the 10^{th} day after immunization blood sample was collected by retro orbital puncture. Hemaggluination titer assay was performed for antibody level determination. Serial dilution of serum and 0.1% bovine serum albumin (100 µl: 100 µl) was prepared in sterile saline. One volume (100 µl) of 0.1% SRBC in saline was added and mixed. They were allowed to settle at room temperature for 90 min till control tube showed a negative pattern (a small button formation). The value of highest serum dilution showing visible hemagglutination was considered as antibody titer.

Carbon clearance test [21]

The mice were divided into 6 groups. Each group consists of 6 animals. Group I (control) was given 1% sodium carboxy methyl cellulose in water (0.3 mL/mouse, orally) for 5 days, Mice in group II-IV were given different concentrations of *Boswellia serrata* Roxb ethanolic extract at doses of 50, 100 and 150 mg/g, p.o., and group VI standard drug (Levimasole 2.5 mg/kg, p.o.) for 5 days. At the end of 5 days, after the gap of 48 h, the mice were injected, via the tail vein, with carbon ink suspension (10 µl/g bw). Blood samples were drawn (in EDTA solution 5 µl), from the retroorbital vein, at interval of 0 and 15 min. A 25 µl sample was mixed with 0.1% sodium carbonate solution (2 mL) and absorbance was measured at 660 nm. The carbon clearance was calculated using the following equation: $(Log_e\ OD_1\text{-}Log_e\ OD_2)/15$, where, OD1 and OD2 are optical densities at 0 and 15 min, respectively.

Enzymatic action

Animals of all groups were sacrificed 24 h. after the last dose. In all groups' animals, spleen were collected after the scarification and washed immediately with ice cold saline to remove blood. Spleen tissues of mice were homogenized (10%) in phosphate buffer (pH 7.4). The homogenate was centrifuged at 12000 g for 20 min at 4°C to obtain supernatant and it was used for the estimation of reduced glutathione (GSH)**, S**uperoxide dismutase (SOD) and Lipid peroxidation (LPO) [22].

Reduced glutathione (GSH)

To the 1 ml of the suspension medium, 5 ml of 0.02 M EDTA was added and then to it 4.0 ml of cold distilled water was added. After mixing it well, 1 ml of 50% trichloroacetic acid (TCA) was added and shaken intermittently for 10 minutes using a vortex mixer. After 10 minutes the contents will be transferred to centrifuge tubes (rinsed in EDTA) and centrifuged at 6000 rpm for 15 minutes. Following centrifugation, 2 ml of the supernatant was mixed with 4.0 ml of 0.4 M Tris buffer (pH 8.9). The whole solution was mixed well and 0.1 ml of 0.01M DTNB was added to it. The optical density (O.D.) was read within 5 min of the addition of DTNB at 412 nm and the results expressed as nmol/ g tissue of glutathione.

Superoxide dismutase (SOD)

To the 0.1 ml supernatant, 1.2 ml of sodium pyrophosphate buffer (0.052 M), 0.1 ml phenazine methosulphate (186 µM), 0.3 ml of nitrobluetetrazolium (300 µM) and 0.2 ml of 750 µM Nicotinamide adenine dinucleotide (NADH) were added. The mixture was incubated for 90 seconds at 30oC. 0.1 ml of glacial acetic acid was added and stirred with 4 ml butanol. The butanol layer was separated after centrifugation at 2000 rpm. Absorbance was taken at 560 nm and expressed as U/mg.

Lipid peroxidation (LPO)

To the 0.2 ml tissue homogenate, 0.2 ml of sodium dodecyl sulfate (8.1%), 1.5 ml of acetic acid (20%) and 1.5 ml of thiobarbituric acid (8%) was added. The volume of the mixture was made up to 4 ml by distilled water and heated on water bath at 95^{o}C for 60 minutes. After cooling 5 ml of butanol: pyridine (15: 1) was added and vortexed. The upper organic layer was separated was absorbance was read at 532 nm. The amount of MDA was calculated from MDA standard curve and expressed as nMole/g tissue.

Determination of Pro-inflammatory cytokine level

The concentrations of TNF- α in the mice serum were determined specific quantitative sandwich ELISA kits.

Statistical Analysis: Data was expressed in Mean ± SD. Statistical comparison between different groups was done by using One Way ANOVA followed by Benferroni's test. $P<0.05$ and $P<0.001$ were considered as levels of significance.

RESULTS

Phytochemical analysis

BSREE exhibited various phytoconstituents as glycosides, flavonoid and phenols as shown in Tables 1 and 2.

Table 1: Results of percentage yield of *Boswellia serrata* Roxb extract

Plant Name	Percentage yield (%)
	Ethanol
Boswellia serrata	17.24

Table 2: Phytochemical analysis of *Boswelliaserrata* Roxb extract.

Test	Ethanolic extract
Test for glycosides	
Borntrager's	+ Ve
Keller–Kiliani	+Ve
Test for alkaloids	
Mayer's	-Ve
Hager's	- Ve
Wagner's	- Ve
Test for saponins	
Froth Test	+ Ve
Test for flavonoids	
Lead acetate	+ Ve
Alkaline reagent	+ Ve
Test for triterpenoids and steroids	
Salkowski's	- Ve
Liebermann–Burchard	- Ve
Test for Tannin and phenolic compounds	
Ferric chloride	+Ve
Lead acetate	+ Ve
Gelatin	+Ve

DPPH Radical scavenging assay

Antioxidant activity of extracts was estimated using DPPH assay and reducing power assay. The IC50 of ethanolic extract of *Boswellia serrata* Roxb was 52.88 and Ascorbic acid was 22.4 respectively. The DPPH Radical scavenging assay of ethanolic extracts of *Boswellia serrata* Roxb was shown in Figure 1. IC 50 = 52.88.

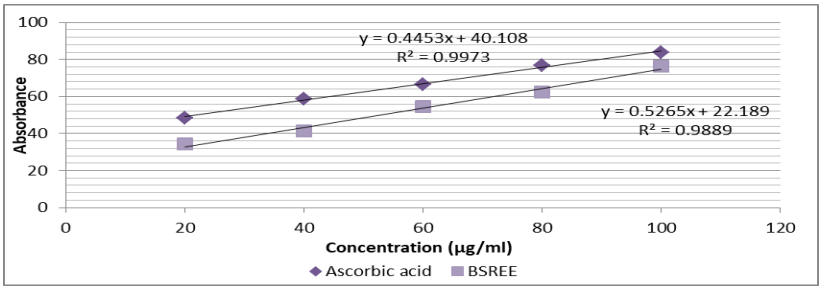

Figure 1: DPPH radical scavenging assay of BSREE and Ascorbic acid.

Reducing Power Assay

The reducing power assay of ethanolic extracts of *Boswellia serrata* Roxb was given in Table 3 and Figure 2.

Table 3: Reducing power assay of ethanolic extracts of *Boswellia serrata* Roxb.

Concentration (µg/ml)	Absorbance	
	AA	BSREE
20	0.202	0.095
40	0.271	0.13
60	0.34	0.181
80	0.401	0.246
100	0.465	0.312
Mean ± SD, n=6		

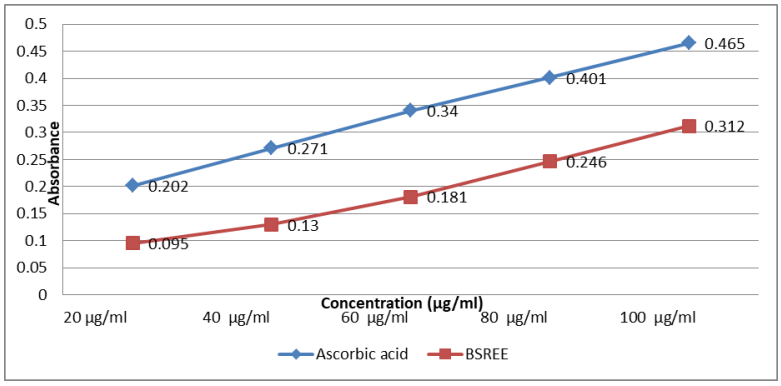

Figure 2: Reducing power assay of BSREE and Ascorbic acid.

Acute toxicity study

The acute toxicity test allowed the estimation of median lethal dose (LD50), which represented the dose that killed 50% of the tested population, which had been used to appreciate the toxicity of the samples. The study also provided useful information concerning the effect of acute exposure of test animals to high doses of extracts under investigation. Treatment of Swiss mice with *Boswellia serrata* Roxb ethanolic extract did not produce treatment-related mortality at the limit test dose (2000 mg/kg), and besides, throughout the 14 days observation period, no significant changes had been discovered in the behavior, such as apathy, hyperactivity, dizziness, vomiting, diarrhea, excessive salivation, loss of fur, anxiety, convulsions, lethargy, and morbidity, among the tested animals. Furthermore, no abnormal changes attributable to treatment had been noticed in body weights and treatment related changes like respiration rate and heart rate. Thus, *Boswellia serrata* Roxb ethanolic extract had been found to be safe at the dose level of 2000 mg/kg and therefore, the LD50 value for oral toxicity had been considered to be more than 2000 mg/kg. So, the dose decided for its immunomodulatory effect was 50, 100 and 150 mg/kg bw.

Effect of BSREE on delayed type hypersensitive reaction

In delayed type hypersensitivity (DTH), test it was observed that BSREE showed significant effect ($p<0.05$) at 50 mg/kg, 100 mg/kg and 150 mg/kg as compared to that of vehicle treated group. Results are mentioned in Table 4. Effect of

extract was significant at 48 hour as well as on 72 hour. Thus, stimulatory effect of BSREE on delayed type hypersensitivity test revealed that extract was having stimulatory effect on T lymphocytes (Figure 3).

Table 4: Effect of *Boswellia serrata* Roxb ethanolic extract at doses of (50, 100 and 150 mg/kg) on delayed type hypersensitivity response in mice

Groups	Treatment	24 hr	48 hr	72 hr
G1	Vehicle treated	1.71 ± 0.420**	1.28 ± 0.547**	0.93 ± 0.434**
G2	BSREE (50 mg/kg)	2.14 ± 0.197**	1.99 ± 0.286**	1.86 ± 0.352^{NS}
G3	BSREE (100 mg/kg)	2.03 ± 0.152**	1.91 ± 0.410**	1.47 ± 0.375**
G4	BSREE (150 mg/kg)	1.94 ± 0.193**	1.80 ± 0.430**	1.11 ± 0.333**
G5	Levamisole (2.5 mg/kg)	1.62 ± 0.519**	1.05 ± 0.276**	0.87 ± 0.402**
G6	SRBC control	3.03 ± 0.183	2.87 ± 0.277	2.52 ± 0.412

Values are presented as MEAN ± SD at n=6, One way ANOVA followed by Bonferroni test, Test extract (BSREE) treated groups were compared with SRBC control group, ** p <0.05 and ^{NS}P>0.001 compared SRBC control group

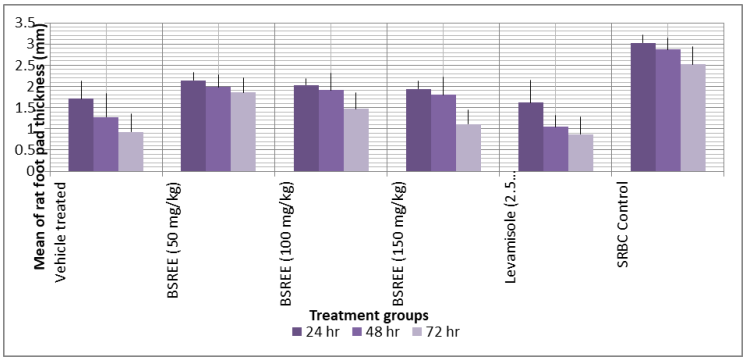

Figure 3: Effect of different concentrations of *Boswellia serrata* Roxb ethanolic extract on the delayed hypersensitivity reactions in mice by measuring paw size. Control, LEV 2.5 mg.kg, *Boswellia serrata* Roxb extract 50 mg/kg bwt; *Boswellia serrata* Roxb extract 100, *Boswellia serrata* Roxb extract 150 mg/kg bw and SRBC Control

Effect of BSREE on Hemagglutination antibody titer

Ethanolic extract was evaluated for its immunomodulatory by using Hemagglutination antibody titer method. BSREE produced significant effect ($p<0.05$) at 50mg/kg, 100 mg/kg and 150 mg/kg as compared to that of vehicle treated group. Observation is mentioned in Table 5. Thus, in HAT test it can be postulated that BSREE showed its effect by modulating B lymphocytes, plasma cells, IgG and IgM involved in humoral immune system (Figure 4).

Table 5: Effect of *Boswellia serrata* Roxb ethanolic extract at doses of (50, 100 and 150 mg/kg) on the of humoral antibody response to SRBC as determined by hemagglutination antibody titers in mice.

Groups	Treatment	Mean Hemagglutination antibody titre (HAT) Units/ µl
G1	Vehicle treated	5.50 ± 0.577 ^{NS}
G2	BSREE (50 mg/kg)	4.00 ± 0.816**
G3	BSREE (100 mg/kg)	5.25 ± 0.500 ^{NS}

G4	BSREE (150 mg/kg)	7.00 ± 0.816^{NS}
G5	Levamisole (2.5 mg/kg)	8.50 ± 0.577**
G6	SRBC control	6.00 ± 0.86
Values are presented as MEAN ± SD at n=6, One way ANOVA followed by Bonferroni test, Test extract (BSREE) treated groups were compared with SRBC control group, p <0.05, NS P>0.001 compared SRBC control group		

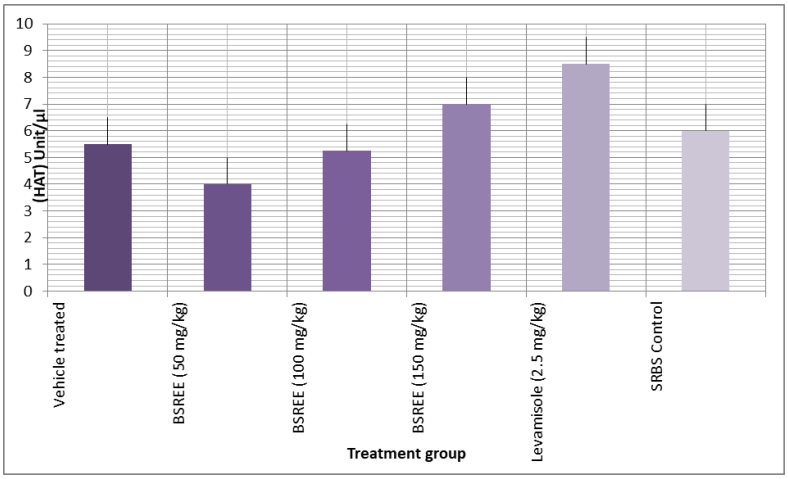

Figure 4: Effect of different concentrations of *Boswellia serrata* Roxb ethanolic extract on the on the of humoral antibody response to SRBC as determined by hemagglutination antibody titers in mice. LEV 2.5 mg.kg, Control, *Boswellia serrata* Roxb extract 50 mg/kg bwt; *Boswellia serrata* Roxb extract 100, *Boswellia serrata* Roxb extract 150 mg/kg bwt and SRBC Control

Effect of BSREE on carbon clearance test

The carbon clearance test was done to evaluate the effect of drugs on the reticulo endothelial system. The reticuloendothelial system (RES) is a diffuse system consisting of phagocytic cells. All doses of BSREE showed significant increase in the phagocytic index when compared to control indicating that there was increase in the clearance of colloidal carbon from the blood after administration of this extract at 50, 100 and 150 mg/kg bw. The effect of BSREE on carban clearance in mice was shown in Figure 5 and Table 6.

Table 6: Effect of *Boswellia serrata* Roxb ethanolic extract at doses of (50, 100 and 150 mg/kg) on Carbon clearance assay in mice

Groups	Treatment	Phagocytic index
G1	Vehicle treated	0.08 ± 0.014
G2	BSREE (50 mg/kg)	0.05 ± 0.010**
G3	BSREE (100 mg/kg)	0.06 ± 0.010**
G4	BSREE (150 mg/kg)	0.09 ± 0.011^{NS}
G5	Levamisole (2.5 mg/kg)	0.08 ± 0.010^{NS}
Values are presented as MEAN ± SD at n=6, One way ANOVA followed by Bonferroni test, Test extract treated groups were compared with normal control group, p < 0.05, NSP>0.001 compared normal control group		

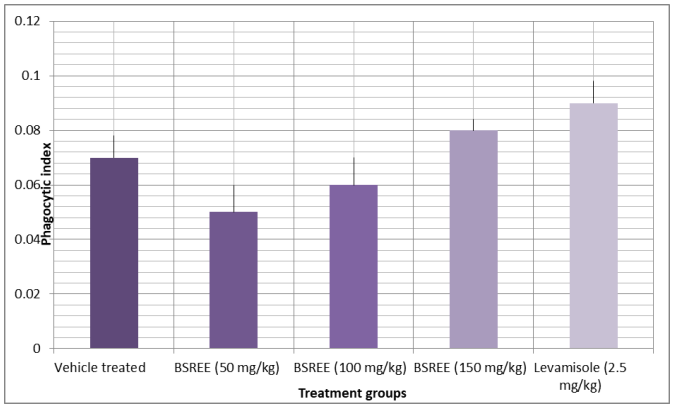

Figure 5: Effect of different concentrations of *Boswellia serrata* Roxb ethanolic extract on the on Carbon clearance assay in mice. LEV 2.5 mg.kg, Control, *Boswellia serrata* Roxb extract 50 mg/kg bwt; *Boswellia serrata* Roxb extract 100, *Boswellia serrata* Roxb extract 150 mg/kg bwt

Quantification of TNF-α

The level of TNF-α was measured by using enzyme linked immunosorbent assay (ELISA). The level of TNF-α is expressed as ng/mg protein using a calibration curve obtained from standard TNF-α (Figures 6-10).

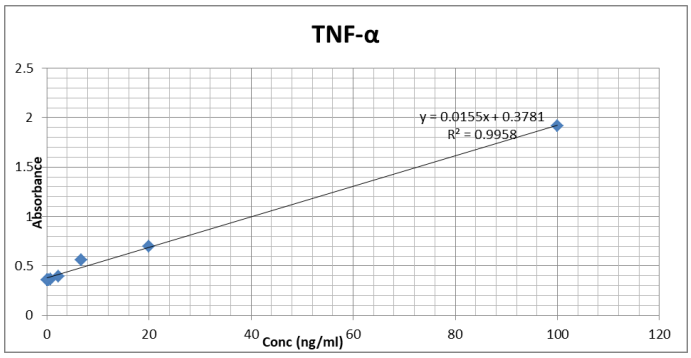

Figure 6: Effect of BSREE on the changes in TNF-α level in control and experimental animals.

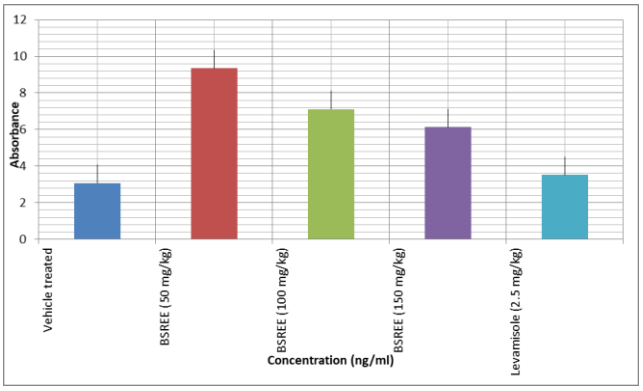

Figure 7: Effect of BSREE on the changes in TNF-α Level in control and experimental animals.

Effect of BSREE on LPO, GSH and SOD

The mice showed an increase (30.72 ± 1.676 nmolMDA/g) in lipid peroxidation level but BSREE treatment, the elevated lipid peroxidation decreased to 13.17 ± 2.049 nmol MDA/g.

The effects on GSH and SOD were dose dependent, the activities of serum GSH and SOD in animals administrated with the BSREE (150 mg/kg) being significantly greater than that in the mice receiving BSREE (50 mg/kg) ($p < 0.05$) respectively (Tables 7, 8 and 9).

Table 7: Effect of BSREE on oxidative stress parameter lipid peroxidation.

Groups	Treatment	nmol/g tissue
G1	Vehicle treated	38.72 ± 1.676
G2	BSREE (50 mg/kg)	20.53 ± 1.536**
G3	BSREE (100 mg/kg)	16 ± 0.568**
G4	BSREE (150 mg/kg)	13.17 ± 2.049**
G5	Levamisole (2.5 mg/kg)	10.53 ± 1.092**
Values are presented as MEAN ± SD at n=6, One way ANOVA followed by Bonferroni test, Test extract treated groups were compared with normal control group, p < 0.05, NSP>0.001 compared normal control group		

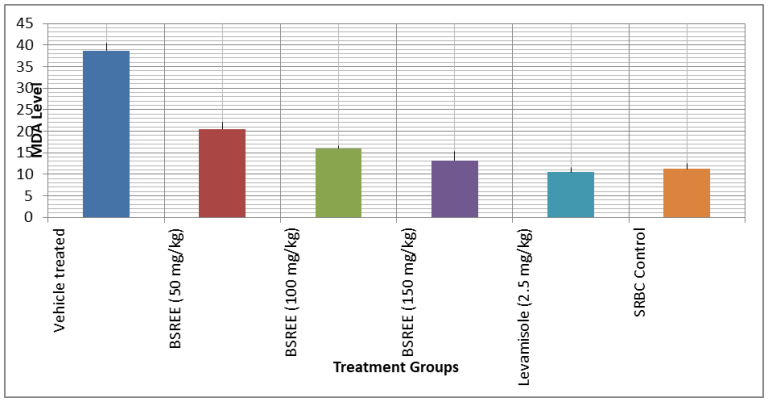

Figure 8: Effect of *In vivo* antioxidant activity of BSREE utilizing LPO assay.

Table 8: Effect of BSREE on oxidative stress parameter superoxide dismutase.

Groups	Treatment	SOD Level Unit/mg tissue
G1	Vehicle treated	84.06 ± 2.696
G2	BSREE (50 mg/kg)	55.34 ± 6.713**
G3	BSREE (100 mg/kg)	62.88 ± 4.135**
G4	BSREE (150 mg/kg)	70.32 ± 3.574**
G5	Levamisole (2.5 mg/kg)	93.99 ± 2.52**
Values are presented as MEAN ± SD at n=6, One way ANOVA followed by Bonferroni test, Test extract treated groups were compared with normal control group, p < 0.05, , NSP>0.001 compared normal control group		

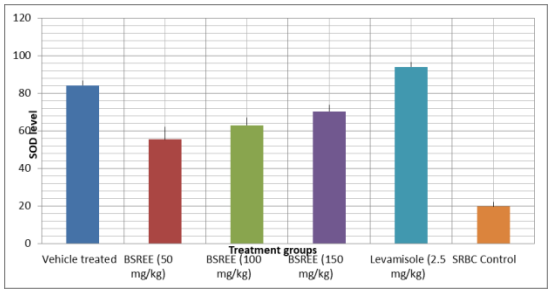

Figure 9: Effect of *In vivo* antioxidant activity of BSREE utilizing SOD assay.

Table 9: Effect of BSREE on oxidative stress parameter reduced glutathione.

Groups	**Treatment**	**GSH nmol/g tissue**
G1	Vehicle treated	2.858 ± 0.019
G2	BSREE (50 mg/kg)	0.611 ± 0.02**
G3	BSREE (100 mg/kg)	1.831 ± 0.112**
G4	BSREE (150 mg/kg)	2.256 ± 0.010**
G5	Levamisole (2.5 mg/kg)	4.098 ± 0.114**
Values are presented as MEAN ± SD at n=6, One way ANOVA followed by Bonferroni test, Test extract treated groups were compared with normal control group, $p < 0.05$, NSP>0.001 compared normal control group		

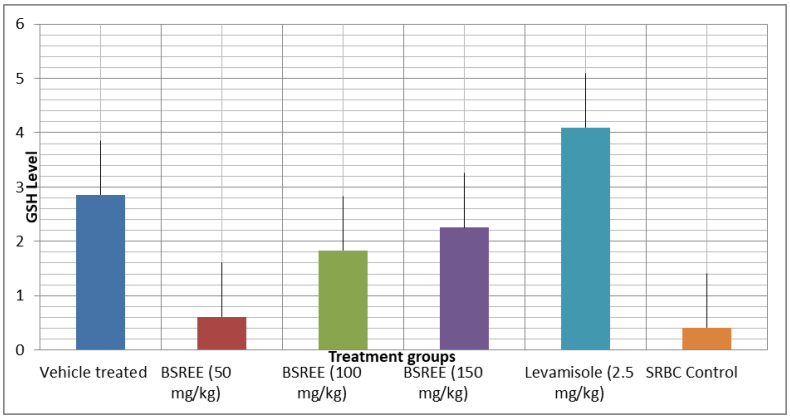

Figure 10: Effect of *In vivo* antioxidant activity of BSREE utilizing GSH assay.

DISCUSSION

The preliminary phytochemical analysis revealed the presence of glycosides, saponin flavonoids, tannins and phenolic compounds. Levamisole is the only known oral allopathic salt used as immunostimulant, which restores the suppressed immune function of B cells, T cells, monocytes and macrophages. *Boswellia serrata* Roxb ethanolic extract (BSREE) produced dose related decrease in delayed type hypersensitivity response at the selected range of doses i.e. 50, 100 and 150 mg/kg. As with humoral antibody titer, the most significant ($P<0.05$) result was observed in mice. The haemagglutination antibody reaction against antigens will be carried out by antibody molecules which are the product of B lymphocytes and plasma cells. This reaction will act as a central role in humoral immune response against different antigens. In the present study, HA titre which is

mediated by IgG and IgM type of immunoglobulins was shown significant inhibition by BSREE 50, 100, 150 mg/kg in humoral immunity. BSREE at a dose of 150 mg/kg produced the maximum increase of antibody formation, respectively which were comparable to levamisole 2.5 mg/kg used as a standard drug, thus indicating BSREE significantly ($P<0.05$) potentiate antibody formation. Phagocytosis represents an important immune defence mechanism in which leukocytes ingest pathgenic microorganisms, malignant cells, tissue debris and inorganic particles (carbon ink). The *in vitro* phagocytosis test was done to evaluate the effect of extract on the reticuloendothelial system (RES). It is a diffuse system containing phagocytic cells. When the colloidal carbon particles are injected directly into the systemic circulation, it is cleared by RES involving phagocytes. BSREE showed remarkable augmentation at 150 mg/kg in the phagocytic index. Glutathione plays a fundamental role in numerous metabolic and biochemical reactions such as DNA synthesis and repair, protein synthesis, prostaglandin synthesis, amino acid transport and enzyme activation. Thus, every system in the body, especially the immune, nervous, and gastrointestinal systems, as well as the lungs, can be affected by the level of glutathione [23]. Superoxide dismutase induces the activation of endogenous system of antioxidant defences which fights against free radicals. It is known that superoxide dismutase plays an important role in the detoxification of superoxide anion and H_2O_2, thereby protecting the cell against free radical induced damage [24]. The inflammatory response in the body is mediated by the proinflammatory cytokines such as tumor necrosis factor alpha (TNF-α). The level and persistence of TNF-α play an important role in determining the behavior of a given factor in immunomodulation. Our result shows that ethanolic extract of *Boswellia serrata* induce cytokine production (TNF-α) in a dose-dependent manner.

CONCLUSION

Based on the findings from the study, the ethanol extract of *Boswellia serrata* increased both the cell-mediated and humoral immune responses in mice. This could be attributed to the different macronutrients, micronutrients and phytochemicals present in the plant. The ethanolic extract of *Boswellia serrata*, therefore, has a potential therapeutic value in several immunosuppressing clinical conditions and hence the reason for its use in local communities to alleviate various disease conditions.

CONFLICT OF INTEREST

All authors have declared that no conflicts of interest exist.

AUTHORS CONTRIBUTIONS

All authors contributed to the manuscript.

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