American Journal of Advanced Drug Delivery

www.ajadd.co.uk

Original Article

In vivo Antioxidant and Immunomodulatory Activity of *Bombax ceiba* Bark - Focusing on its Invigorating Effects

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Date of Receipt- 25/11/2013
Date of Revision- 30/11/2013
Date of Acceptance- 02/11/2013

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ABSTRACT

Objective: The present study evaluated the antioxidant and immunomodulatory activity of methanol extract of the bark of *Bombax ceiba* in normal and immunosuppressed mice models.
Materials and Methods: *In vivo* immunomodulatory and antioxidant activity of *Bombax ceiba* methanol extract was evaluated by assessing its effect on Hemagglutinating antibody (HA) titer, delayed type of hypersensitivity (DTH) response, hematological profile (Hb, WBC, RBC), lipid per oxidation (LPO), reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and cytokine release.
Results: The animals treated with *Bombax ceiba* methanol extract showed increase in antibody titer values 11.2 ± 0.30 and 13.1 ± 0.27 at 150 and 300 mg/kg (p.o.) dose and DTH reaction induced by SRBC was also found significant ($P < 0.001$). Also it caused increase in hematological profile, GSH, SOD, CAT activity and significantly decreased LPO levels in cyclophosphamide-induced immunosuppressed mice. The extract treated animals showed a significant up regulation of (IL-6 and TNF-α) cytokines in comparison to control group animals.
Conclusion: These findings suggested that the methanol extract of *Bombax ceiba* possessed promising immunostimulant properties which could be ascribed, in part, to its anti-oxidant capacity.

Keywords: Lipid peroxidation; Immunoadjuvant; Cytokine; Cyclophasphamide.

INTRODUCTION

Our daily rhythms can have a profound effect on immune response through hormonal modifications. Immunological diseases are growing at epidemic proportions that require aggressive and innovative approaches to the development of new treatments. Natural product resources provide excellent raw material for the discovery and development of novel immunomodulatory compounds. Immunomodulatory medicinal plants are comparatively a recent concept in pharmacognosy. In many of the diseased conditions, immune response is impaired. Hence, to maintain a disease-free state, modulation of immune response, by either its stimulation or suppression, can be a helpful therapy. Immunomodulators can provide supportive therapy to the chemotherapy.[1] Immunomodulators can regulate the cytokine production such as tumor necrosis factor, interleukins and interferons and these cytokines may, in turn activate T-cells or NK cells.[2-4] Many studies have been performed to assess the potential utility of natural products as immunomodulatory agents to enhance host responses to disease/infection or to ameliorate immune based pathologies (i.e., inflammation, autoimmune associated diseases etc.).

Immunoadjuvants are used to enhance the efficacy of vaccines and therefore could be considered specific immune stimulants. Immunoadjuvants hold the promise of being the true modulators of immune response. It has been proposed that they can be exploited as selectors between cellular and humoral helper T1 (Th1) and T2 cells (Th2), immunoprotective, immune-destructive and reagenic versus IgG type immune responses-posing a real challenge to vaccine designers.[5,6]

Bombax ceiba (syn. *Bombax malabaricum*) is an important medicinal plant of tropical and subtropical India commonly known as Silk Cotton Tree or Semal.[7] It is a tall deciduous tree, with straight butteressed trunk and wide spreading branches. Almost every part of this plant is used as medicine for curing maximum number of ailments. Its bark is mucilaginous, demulcent and emetic, and is used in healing wounds, bark paste is good for skin eruptions.[8,9] Cotton tree has been used extensively for treatment of some diseases like inflammation[10], algesia[11], hepatotoxicity[12] and hypertension, as well as for antiangiogenic and antioxidant activities.[13] Young roots of the plants have been reported to be useful in diarrhoea, dysentery, urinary troubles, gynecological problems, bladder disorders, heart diseases, debility, diabetes and impotence.[14,15] Bark contains lupeol, saponins, tannins, gums and 4, 5, 7- trihydroxy-flavone-3-O-β-D-glucopyranosyl (1-4)-α-L-rhamno-pyranoside.[16] In other studies secondary metabolites like triterpenoids obtained from plants influenced both cellular and humoral immune responses in rats and mice and it has been reported that polyphenols, flavone are effective scavengers of free radicals, and also helps in the modulation of immune functions.[17] However, there is no scientific report available in the literature against the effect of these phytoconstituents enriched extract in immune modulation. Therefore, the present study was undertaken to assess the *in-vivo* antioxidant and immune-modulatory activity of *Bombax ceiba* bark extract against cyclophosphamide-induced immunosuppression.

MATERIALS AND METHODS

Plant material Collection and authentication

The fresh bark of the *Bombax ceiba* was collected from the field area of Bahraich, district U.P. India, during the month of

March, 2011. The plant material was authenticated by Dr. A. K. S. Rawat, Scientist and Head, Pharmacognosy and Ethnopharmacology Division, National Botanical Research Institute Lucknow, India with specification no. NBRI-SOP-202, CIF-RB-2-121.

Preparation of extract

Shade-dried and coarsely powdered 100 g powder from stem bark of *Bombax ceiba* was soaked in 500 ml of methanol [methanol/drug mass ratio 5:1]. It was kept at room temperature for 48 hours with intermittent mixing. Methanol extract of plant (MEBc) obtained after 48 hr of soaking was filtered using Whatman Qualitative Grade-1 filter paper and the resultant filtrates were concentrated under reduced pressure and finally vacuum dried.

Chemo profiling of MEBc

Preliminary phytochemical screening of extract was done as per WHO recommended guidelines for standardizations.[18] The qualitative analysis of the MEBc was performed on HPTLC comprising of densitometer (CAMAG Model-3 TLC scanner) equipped with win CATS 4 software, semi automatic sampler (Linornat-5) and documentation was carried out by CAMAG PROSTER 3 at 366 nm (CAMAG, Berlin, Germany).

Drugs

Cyclophosphamide (Endoxan from Cadila Healthcare limited, India.) 50 mg / kg b.w was used.[19] Plat extract doses used in the present study were 150 and 300 mg/kg.[11, 19] All drugs were dissolved in pyrogen-free isotonic saline. Suspension of the MEBc was prepared in carboxy methyl cellulose (CMC, 0.3%) using pyrogen-free isotonic saline.

Test animals

Healthy Swiss albino mice (25-30 g) of either sex were selected for the study. The animals were housed under standard 12hr: 12hr light/dark cycles in the animal house of Integral University, and were fed on commercial diet (Hindustan lever pellets, Bangalore) and water *ad libitum*. The animals were acclimatized to the laboratory conditions before testing them. Each animal was used once. The experiments were performed between 10.00 and 16.00 h. The experimental protocol has been approved by the institutional ethical committee with approval no. IU/Pharm/PhD./CPCSEA/12/06.

Antigenic materials

For the present study, the antigenic material used was sheep RBCs (SRBC). Fresh blood was collected from sheep sacrificed in the local slaughter house. It was mixed with Alsever's solution in 1:1 proportion and was stored at 4ºC in refrigerator. During the experimentation, adequate amount of blood was taken from the above stock solution (i.e. SRBCs, stored in Alsever's solution) and was allowed to stand at room temperature. It was washed three times with pyrogen free normal saline (0.9% w/v NaCl). The RBC count of this suspension was determined by hemocytometer using Neubauer Chamber. The known amount of RBCs (0.5 x 10^9cells / ml / 100 g) was injected intraperitoneally to the mice as an antigenic challenge.

Dosing schedule

Animals were divided into six groups (I-VI). Each group comprised of a minimum of six animals. Group I (control) received normal saline for 7th consecutive days, group II (Cyclophasphamide) animals were injected with a single dose of CP on 6th day of initiation of experiment, group III (Plant extract BC_1) animals and group IV (Plant extract BC_2) animals were administered with

plant extract for 7th consecutive days. Group V animals (Plant extract BC_1+ CP) and group VI animals (Plant extract BC_2+ CP), were given plant extract treatment for 7 th days with a single injection of CP ip on 6th day. For humoral response animals of all groups will be challenged with 0.2 ml of 10% SRBC ip on 5th day.

Humoral immune response model

Measurement of antibody titer by hemagglutination reaction was performed by using method of Bin-Hafeez et al. (2001) with some modification. The mice were lightly anesthetized with anesthetic ether. A fine capillary was gently inserted into the lower angle of eye at 45° and the blood was obtained from retro-orbital plexus. The blood was collected into vial and centrifuged for separating serum. The serum of mice was used for determination of hemagglutination titer. Micro titration plate having 96 cups was used for carrying out titration. Serial two fold dilutions of serum were prepared. To each cup 25 ± l µl of 1% v / v SRBC was added. The plate was incubated at 37ºC for 1 h and then was observed for agglutination. The antibody titer was expressed in terms of maximum dilution, which gave positive hemagglutination reaction.[19]

Cellular immune response model

Cell mediated immune response was assessed by footpad reaction test. On 7th day, after measuring volume of footpad of both legs, SRBC (0.025 x 10^9 cells) were injected in right paw and 0.025 ml of saline was injected in left paw. On 8th day after 24 h, the paw volume was measured again to check the increase or decrease in volume. The increase in paw volume was considered as an index of cell mediated immunity.[20]

Blood Parameters

The blood withdrawn from the above antigenically challenged mice were used to estimate the hematological parameters like hemoglobin (Hb), RBC and WBC by usual standardized laboratory method.[21]

Body weight and Relative organ weight Determination

Animals of all groups were sacrificed 24 h. after the last dose. Body weight gain and relative organ weight (organ weight / 100 g of body weight) of kidney, liver, and spleen were determined for each animal.[19]

Assessment of antioxidant parameters

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 LPO and reduced glutathione (GSH).[22,23]

Assay of TBARS

1 ml of the suspension medium was combined with 0.5 ml of 30% TCA and to this 0.5 ml of 0.8% TBA reagent was added. The tubes were then be covered with aluminium foil and kept in shaking water bath for 15 minutes. After 15 minutes tubes were taken out and kept in ice-cold water for 30 minutes. The resultant precipitate was removed by centrifugation at 3000 rpm for 15 minutes. The absorbance of the supernatant was read at 540 nm at room temperature against appropriate blank. The concentration of the TBARS was determined using a molar extinction coefficient of 1.56×10^5/M/cm.[22]

Assay of Glutathione

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 nmoles of glutathione of protein.[23]

Assay of SOD

The supernatant was assayed for SOD activity by following the inhibition of pyrogallol autoxidation. 100 µl of supernatant was added to Tris HCl buffer (pH 8.5). The final volume of 3 ml was adjusted with the same buffer. At least 25 µl of pyrogallol was added and changes in absorbance at 420 nm are recorded at 1 minute interval for 3 minutes. The increase in absorbance at 420 nm after the addition of pyrogallol was inhibited by the presence of SOD. The units of the SOD activity which were determined were expressed in terms of milligrams of the total protein.[24]

Assay of Catalase

Spleen tissue was homogenized in 50 mM/L potassium phosphate buffer with a ratio of 1:10 w/v. The homogenate was centrifuged at 10,000 rpm at 4º C in a cooling centrifuge for 20 minutes. Catalase activity was measured in supernatant obtained after centrifugation. Supernatant (50 µl) was added to cuvette containing 2.95 ml of 19 mM/L solution of H2O2 prepared in potassium phosphate buffer. Change in optical density was measured at 240 nm by kinetic method with 1 min interval. The units of the CAT activity which were determined were expressed in terms of nmol H_2O_2/mg protein.[25]

Determination of Proinflammatory cytokine level

The concentrations of TNF- α and IL-6 in the mice serum were determined specific quantitative sandwich ELISA kits according to the instruction of the manufacturer purchased from eBioscience and Cayman Chemical USA.[26]

Statistical Analysis

Data were statistically analyzed using student's t-test to determine significant differences in data of various groups; *P* values less than 0.05 were considered significant. The values are expressed as means ± SEM.

RESULTS

Standardization of Extract

The preliminary phytochemical investigation of the extract of *Bombax ceiba* showed the presences of Alkaloids, phytosterols, flavonoids, carbohydrates, Phenolic compound, tannins and glycosides. The extract was analyzed qualitatively by HPTLC. Finger printing studies on methanol extract showed presence of various phytoconstituents with different intensities, with their respective Rf values (Figure-1) The plate was developed in pre-saturated horizontal chamber with solvent system comprising of n-butanol: Acetic acid: water (6:1:2). The plates were visualized under UV at 366 nm.

Hemagglutinating antibody (HA) titer

The HA titer was used to assess humoral immune response. The CP treatment produced decrease in the HA titer after 2 hr incubation with SRBCs (Table-1). Administration of both the tested doses (150 and 300 mg/kg, respectively) produced a significant increase in HA titer as evident from hemagglutination after incubation of the serum with SRBCs. The increase was found highly significant. Thus, the plant extract

showed protective effects on humoral immunity.

Delayed type hypersensitivity (DTH) reactions

The cell-mediated immune response of *B. ceiba* bark extract was assessed by DTH reaction, i.e. foot pad reaction, as shown in table-1, the test extract produced a significant, dose-related increase in DTH reactivity in mice. Increase in DTH reaction in mice in response to cell dependent antigen revealed the stimulatory effect of *B. ceiba* bark extract on T cells.

Effect on Relative organ weight

CP injection caused a prominent reduction in relative organ weight (kidney, liver, and spleen) of the mice (Table-2) recovery was also observed significant in each organ.

Effect on Hematological parameters

Cyclophosphamide at the dose of 50 mg / kg, i.p. caused a significant reduction in the hemoglobin, RBCs and WBCs (Table-3). Combined treatment of cyclophosphamide and bark extract (150 and 300 mg/kg) restoration of bone marrow activity as compared with cyclophosphamide alone treated mice was observed.

Effect of B. ceiba bark extract on LPO and levels of antioxidant enzymes

The oxidative stress marker studies revealed (Figure-2) that the administration of cyclophosphamide significantly increased the levels of LPO, decreased the activities of SOD, CAT and reduced the content of GSH as compared to the control group. The *B. ceiba* bark extract showed a moderate effect on LPO, SOD, CAT, and GSH. The cyclophosphamide treatment along with *B. ceiba* bark extract decreased TBA reactive products as compared to the cyclophosphamide-exposed group. Moreover, a significant elevation in the CAT and GSH content was also observed in comparison to cyclophosphamide-treated animals. SOD activity also increased when treated with *B. ceiba* bark extract as compared to the cyclophosphamide control group.

Effect of test drugs on proinflammatory cytokine

Effect of test drugs on proinflammatory cytokine production showed that the extract treated animals showed significant up regulation of (IL-6 and TNF-α) cytokines in a dose-dependent manner in comparison to normal animal. (Figure-3) While levels of TNF-α and IL-6 were decreased by the treatment with cyclophasphamide which was then significantly increased in comparison to model control animals, by the administration of test extract along with cyclophasphamide.

DISCUSSION

Considering the structural data, we could state that *Bombax ceiba* posses the immunostimulatory principles. Many of the presently available immunomodulators such as levamisole, glucans and teleronesz are not free from side effects, which include fever, neutropenia, leucopenia and allergic reactions. Hence, screening for new immunomodulators is an urgent need. Immunomodulatory agents of plant origin enhance the immune responsiveness of the organism against a pathogen by activating the immune system.

Bombax ceiba has ability to modulate humoral immune responses by acting at various levels in immune mechanism such as antibody production, release of mediators of hypersensitivity reactions, and tissue responses to these mediators in the target organs. In our study, foot volume was enhanced after *Bombax ceiba* treatment, suggesting cell-mediated immune enhancement. Cell-mediated immunity (CMI)

involves effecter mechanisms carried out by T lymphocytes and their products (lymphokines). The CMI responses are critical to defend against infectious organisms, infection of foreign grafts, tumor immunity and delayed type hypersensitivity reactions. Humoral antibodies that are capable of killing free tumor cells in blood and in serosal cavities have been suggested to play a very important role in cancer. Both experimental and clinical results have demonstrated an apparently paradoxical effect of CP on the tumor-host immune response. The better anti-tumor effect of CP depends on the larger dose of CP administered. However, along with a reduction of the tumor mass, large doses of CP usually bring an impairment of the host defense mechanisms, leading to immunosuppressive and cytotoxic effects.[27]

In the present study, *Bombax ceiba* exhibited beneficial actions on the specific and nonspecific immunity of immune-suppressed mice at the optimal dose. The actions of CP are primarily directed toward the depletion of T/B lymphocytes and the deficiency of macrophages.[27] A significant increase in white blood cell count was observed in methanol extract- treated mice as compared with cyclophosphamide treatment alone. Extract significantly ameliorated the RBC count, hemoglobin and also restored the suppressive effects induced by cyclo-phosphamide. Toxicities of cyclo-phosphamide include the suppression of white blood cells, RBC, Hb, nausea, vomiting, gonadal atrophy, liver, renal, and bladder injury. Significant improvements were found in relative organ weights of kidney, liver, and spleen; therefore, *Bombax ceiba* could be suggested for the drug-induced immunopathy in the organs.

The present study had shown that the administration of cyclophosphamide not only impair the immune responses but also produce oxidative stress in mice. In view of this, it appeared that cyclophosphamide which is a strong generator of superoxide radicals might impair the immune response through oxidative stress. It is further observed that administration of *B. ceiba* bark extract prevented the cyclophosphamide-induced changes of immunological and oxidative stress parameters. Hence, the immunomodulatory effect of *B. ceiba* bark extract may be subsequent to the antioxidant activity which it possesses.

The inflammatory response in the body is mediated by the proinflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6). The level and persistence of TNF-α and IL-6 cytokines play an important role in determining the behavior of a given factor in immunomodulation. IL-6 plays a key role in host immune responses, such as acute protein synthesis, and the maintenance of homeostasis also acts as both a pro-inflammatory and anti-inflammatory cytokine.[28] Our result shows that test drugs induce cytokine production (including IL-6 and TNF-α) in a dose-dependent manner.

CONCLUSION

In conclusion this study shows that the *B.ceiba* bark extract could possibly have both antioxidant and immunostimulatory capabilities. The reactive oxygen species and hyper immune activation are thought to be associated with the pathogenesis of chronic diseases such as inflammatory diseases and HIV/AIDS, and HIV infected individuals have impaired antioxidant defense, the inhibitory effect of the *B. ceiba* bark extract on free radicals and humoral immune response stimulation may partially explain why the *B. ceiba* bark extract is beneficial in ameliorating disease conditions. Protection of immune system by dietary antioxidants may play an important role in preserving the immune function and achieving healthy

ageing. The accruing knowledge from this research is opening up new avenues for targeting cytokines and their receptors, or subunits shared by cytokines or their receptors, to treat undesirable inflammatory conditions. Further studies on the specific mechanism of action of *B. ceiba* bark extract and its semisynthetic derivatives, in order to establish its therapeutic potential for the prevention of immune diseases are required.

REFERENCES

1. Kokate CK, Prohit AP, Gokhale JB. Pharmacognosy. 3rd ed. Pune: Nirali Publishers: 1998. 14 p.
2. Visser D, Eichten KA, Coussens LM. Paradoxical roles of the immune system during cancer development. Nature. 2006; 6: 24–37.
3. Kanjoormana AM, Girija K. Immuno-modulatory activities of Punarnavine, an alkaloid from *Boerhaavia diffusa*, Immunopharmacol Immunotoxicol 2009; 31(3): 377–387
4. Upadhyay SN. Immunomodulation. Narosha publishing house: New Delhi 1997; p. 98–110.
5. Alfons B, Patrick M. Modes of action of Freund's adjuvants in experimental models of autoimmune diseases. *J Leukoc Biol.* 2001; 70:849-60.
6. Kumar D, Arya V, Kaur R, Bhat ZA, Gupta VK, Kumar V. A review of immunomodulators in the Indian traditional health care system. *J Micro, Immuno and Inf.* 2012; 45:165-184.
7. Nadkarni KM. Indian Materia Medica. Popular Prakashan 1976. p. 43.
8. Varier PK, Nambiar VPK, Ramankutty C. Indian Medicinal Plants – A Compendium of 500 species. Orient Longman Publishing, Kerala 1997; p. 289–92.
9. Warrier PK. Indian Medicinal Plants a compendium of 500 species, Orient Longman private limited Chennai 1994. Vol. 2, p. 224–89.
10. Buckingham J. Dictionary of natural products. Landon: Champan and Hall scientific data division 1992. p. 36–8.
11. Saleem R, Ahmad SI, Ahmad M, et al. Hypotensive activity and toxicology of constituents from *Bombax ceiba* stem bark. *Biol Pharm Bull* 2003; 26:41–6.
12. Saleem R, Ahmad M, Hussain SA, et al. Hypotensive, hypoglycaemic and toxicological studies on the flavonol C-glycoside shamimin from *Bombax ceiba*. *Planta Med*. 1999; 65(4):331–4.
13. Vieira TO, Said A, Aboutabl E, et al. Antioxidant activity of methanolic extract of *Bombax ceiba*. Redox Report 1999; 146:41–6.
14. Jain V, Verma SK, Katewa SS. Myths-traditions and fate of multipurpose *Bombax ceiba* L. An appraisal, *Ind j traditional knowl*. 2009; (4):638–44.
15. Katewa, SS, Jain A. Traditional Folk Herbal Medicines. Apex Publishing House, Udaipur 2006; p. 64–5.
16. Rastogi and Mehrotra, Compendium of *Indian Medicinal plants*, Vol. 2, 1990. PID, New Dehli p.104
17. Badria A. Farid, Mikhaeil R. Botros, Maatooq T. Galal, and Amer M. A. Mohamed. Immuno-modulatory Triterpenoids from the Oleogum Resin of *Boswellia carterii* Birdwood. 2003, p. 505-516.
18. World Health Organization (WHO). Quality control guidelines for medicinal plant materials. 1998. p. 111.
19. Hafeez BB, Ahmad Iqbal, Haque Rizwanul, Raisuddin S. Protective effect of *Cassia occidentalis* L. on cyclophosphamide-induced suppression of humoral immunity in mice *J Ethno-pharmacology* 2001;75:13-18.
20. Joharapurkar AA, Zambad SP, Wanjari MM, Umathe SN. In vivo evaluation of antioxidant activity of alcoholic extract

of *Rubia cordifolia linn* and its influence on ethanol-induced immunosuppression. *Ind J Pharmacol*. 2003; 35:232-236.
21. Makare N, Bodhankar S, Rastogi V. Immunomodulatory activity of alcoholic extract of *Magnifera indica* L. in Mice. *J Ethnopharmacol*. 2001; 78: 133:7.
22. Das D, Banerjee RK. Effect of stress on the antioxidant enzymes and gastric ulceration. Mol Cell Biochem 1993; 1125: 115–125.
23. Ellman GI. Tissue sulphhydryl groups. *Arch Biochem Biophys* 1959; 82: 70–77.
24. Marklund S, Marklund G. Involvement of the super oxide anion radical in the auto oxidation of pyrogallol and a convenient assay for super oxide dismutase. *Eur J Biochem* 1974; 47:469-74.
25. Clairbone A. Assay of Catalase. In: Greenwald RA, ed. Hand book of method of oxygen free radical research; Boca Raton, Fla; CRC press; 1985; 283-84.
26. Yang Yi, Sen TL, Ming WZ, Rui FZ, Yuan YD, Bao Y, Zhen CW. Immunomodulatory Activity of Polysaccharide-Protein Complex of Longan (*Dimocarpus longan* Lour.) Pulp, Molecules 2011, *16*, 10324-10336.
27. Matar P, Rozados VR, Gonzalez A.D, Dlugovitzky DG, Bonfil RD, Scharovsky OG. Mechanism of antimetastatic immunopotentiation by low-dose cyclophosphamide. *Eur. J. Cancer* 2000, 36, 1060-1066.
28. Liu CF, Tseng KC, Chiang SS, Lee BH, Hsua WH, Pana TM. Immuno-modulatory and antioxidant potential of *Lactobacillus* exo polysaccharides. *J Sci Food Agric*. 2011.

Table 1. Effect of *Bombax ceiba* (BC) extract on humoral immune response and delayed type hypersensitivity response

Group	Treatment	Mean antibody titer [a] (in terms of rank of cups of titer plate) ± SEM	Mean of right foot pad thickness [a] (mm) ± SEM
Group I	Control (Normal saline)	9.25 ± 0.30	0.80 ± 0.06
Group II	Normal saline + CP	3.17[b] ± 0.16	1.078[b] ± 0.03
Group III	BC 150 mg/kg	11.2[c] ± 0.30	1.16[c] ± 0.26
Group IV	BC 300 mg/kg	13.1[e] ± 0.27	1.195[c] ± 0.27
Group V	BC 150 mg/kg + CP	6.83[d] ± 0.30	1.097 [d]± 0.23
Group VI	BC 300 mg/kg + CP	8.5[d] ±0.22	1.37 [d] ± 0.16

[a]Values are expressed as mean ± SEM of 6 mice, [b]$P<0.001$ Statistical significance versus Group I, [c]$P<0.01$ Statistical significance versus Group I, [d]$P<0.001$ Statistical significance versus Group II, [e]$P<0.05$ Statistical significance versus Group I

Table 2. Effect of *Bombax ceiba* extract on Relative organ weight

Group	Treatment	Relative organ weight [a] (g) ± SEM		
		Liver	Kidney	Spleen
Group I	Control (Normal saline)	5.14±0.01	1.367±0.45	0.622±0.05
Group II	Normal saline + CP	4.82±0.17[b]	1.115[b] ±0.26	0.334[b] ±0.06
Group III	BC 150 mg/kg	5.21[c] ±0.05	1.225[c] ±0.27	0.511[c] ±0.11
Group IV	BC 300 mg/kg	5.25[e]±0.05	1.391[c]±0.46	0.620[f] ±0.08
Group V	BC 150 mg/kg + CP	4.85[d]±0.1	1.31[d] ±0.35	0.452[d] ±0.07
Group VI	BC 300 mg/kg + CP	4.92[d] ±0.12	1.30[d] ±0.02	0.552[d] ±0.11

[a]Values are expressed as mean ± SEM of 6 mice, [b]$P<0.001$ Statistical significance versus Group I, [c]$P<0.01$ Statistical significance versus Group I, [d]$P<0.001$ Statistical significance versus Group II, [e]$P<0.05$ Statistical significance versus Group I, [f]$P<0.01$ Statistical significance versus Group II.

Table 3. Effect of *Bombax ceiba* extract on Hematological parameters[a]

Group	Treatment	RBC (× 10^6/mm^3)	WBC (× 10^3/mm^3)	Hb (g/dl)
I	Control-Normal saline	9.26±0.60	6.26±0.40	13.76±0.48
II	Normal saline + CP	9.22±0.28	1.52±0.29 [b]	12.52 ±0.78 [b]
III	BC 150 mg/kg	9.64±0.34	7.98±0.70 [c]	13.71 ± 0.92 [c]
IV	BC 300 mg/kg	9.59±0.47	8.21±0.38 [c]	14.01 ± 0.51 [c]
V	BC 150 mg/kg + CP	9.35±0.44	3.52±0.43 [d]	12.67 ± 0.28 [d]
VI	BC 300 mg/kg + CP	9.40±0.31	4.31±0.29 [d]	12.73 ± 0.31 [d]

[a]Values are expressed as mean ± SEM for 6 mice, [b]$P<0.001$ Statistical significance versus Group I,[c]$P<0.01$ Statistical significance versus Group I,[d]$P<0.001$ Statistical significance versus Group II, RBC(million/mm^3), WBC(thousand/mm^3), hemoglobin(g/dl)

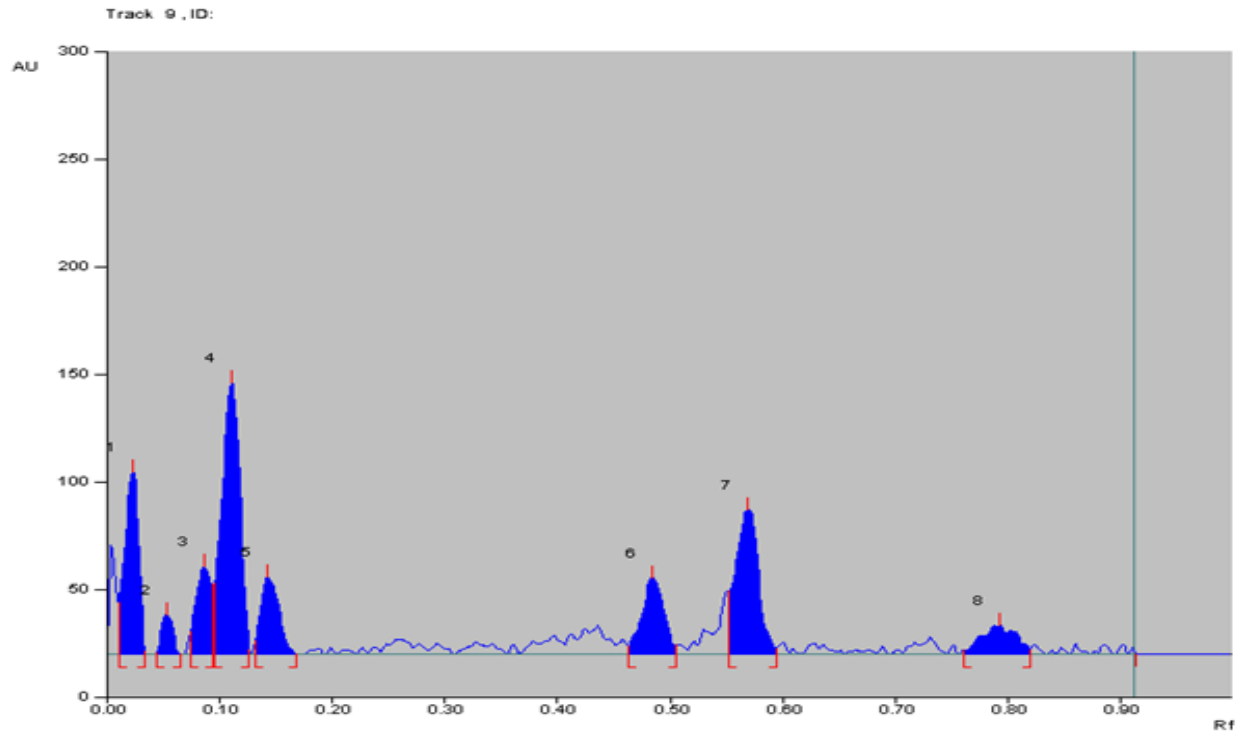

Figure 1. HPTLC Finger printing of methanol extract of *Bombax ceiba* bark

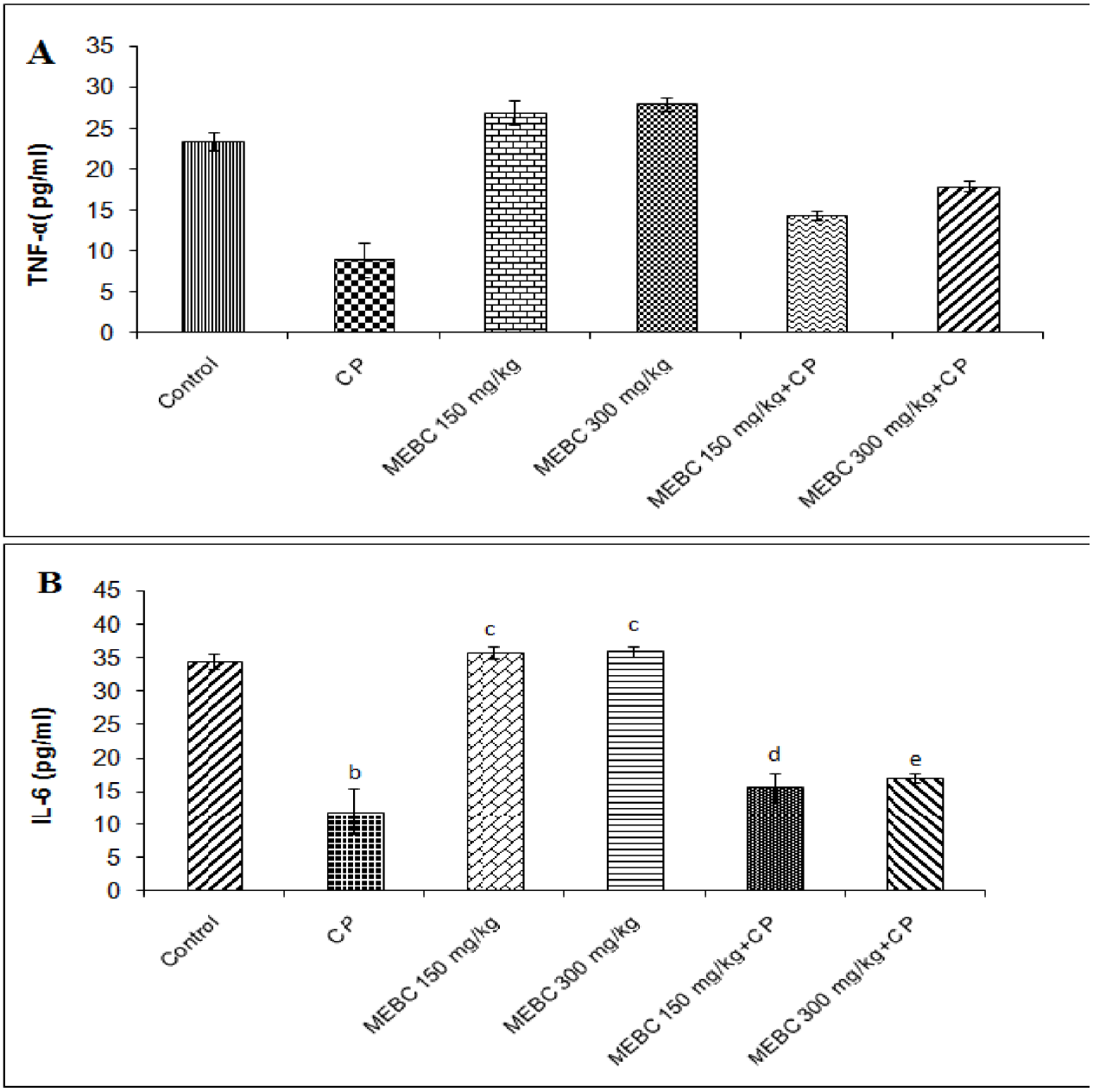

Figure 2. Effect of *B.ceiba* methanol extract (MEBc) on Proinflammatory cytokine level, CP= Cyclophaphamide, MEBC= Methanol extract of *B.ceiba*. (A) Tumor necrosis factor-α (TNF- α), (B) Interleukin-6 (IL-6). Concentration was expressed in pg/ml. Data were expressed as the mean ± SEM of n = 6 animals per group. [b]P<0.05 Statistical significance versus Group I. [c]*P*<0.01 Statistical significance

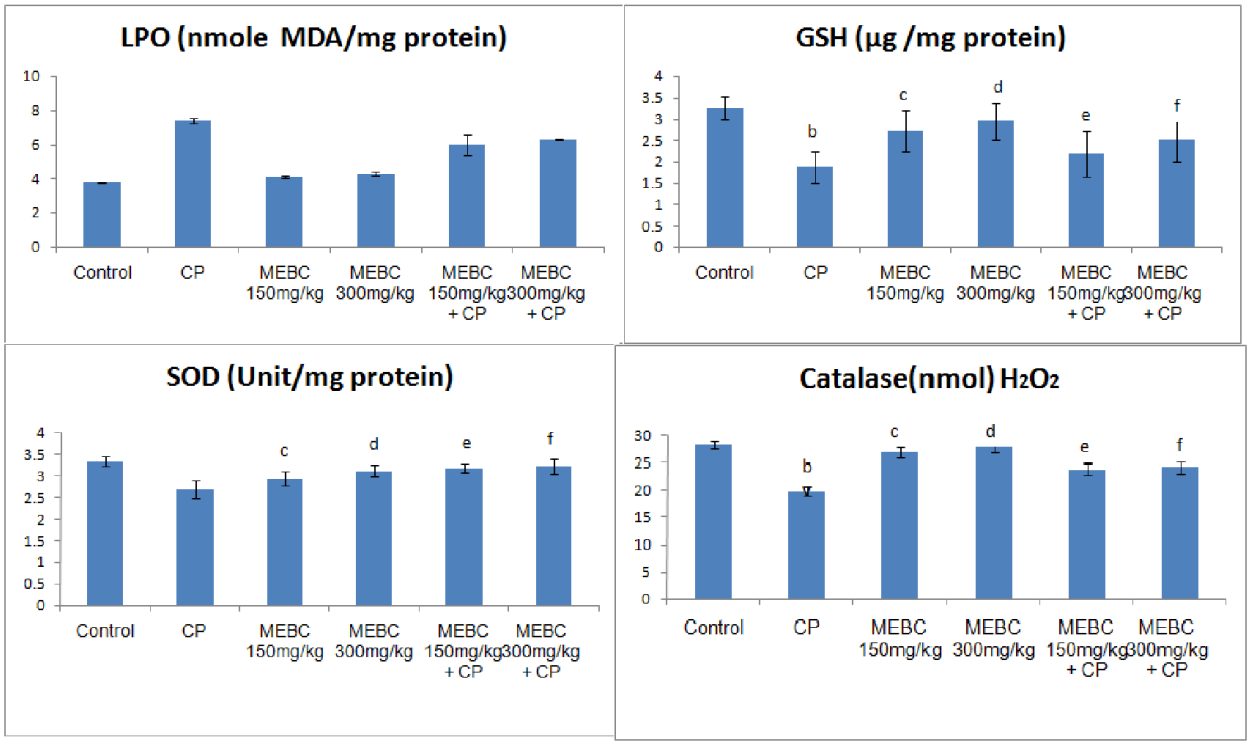

Figure 3. Effect of *Bombax ceiba* extract on Oxidative stress parameters, BC= *Bombax ceiba*, CP=cyclophosphamide, LPO=lipid peroxidation, SOD=superoxide dismutase, GSH=reduced glutathione, CAT=catalase, [a]Values are mean ± SEM of 6 mice, [b]P<0.05 Statistical significance versus Group I, [c]*P*<0.01 Statistical significance versus Group I, [d]*P*<0.001 Statistical significance versus Group I, [e]*P*<0.01 Statistical significance versus Group II, [f]*P*<0.001 Statistical significance versus Group II