



Original Research Article (Experimental)

## Immunomodulatory activities of *Acacia catechu*, a traditional thirst quencher of South India

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## ABSTRACT

**Background:** *Acacia catechu* has been widely used in Ayurveda for treating many diseases. Its heartwood extract is used in asthma, cough, bronchitis, colic, diarrhea, dysentery, boils, skin afflictions, sores and for stomatitis. The decoction of heartwood is used for drinking purpose in southern part of India especially in Kerala.

**Objective:** The current study was carried out to evaluate immunomodulatory effects of heartwood extracts of *A. catechu* in Swiss albino mice.

**Material and methods:** *In vivo* immunomodulatory activity was analyzed by hemagglutinating antibody (HA) titer, plaque forming cell assay and delayed type hypersensitivity (DTH). *In vitro* immunomodulatory potential of the extracts was studied using peritoneal macrophages and splenocytes from mice. Effect of extracts on phagocytic activity of macrophages was analyzed by nitroblue tetrazolium (NBT) reduction assay and cellular lysosomal enzyme assay. Anti-inflammatory activity was studied by nitric oxide (NO) assay and production of TNF- $\alpha$  and IL-10.

**Results:** A dose dependent increase in antibody titer was observed with extracts treatment. Treatment with extracts produced an enhancement in the number of antibody producing cells in the spleen. DTH reaction was significantly decreased with extracts treatment. An increased phagocytic response was shown by peritoneal macrophages on treatment with the extracts as evidenced by its effect on NBT reduction and cellular lysosomal enzyme activity. The extracts inhibited the release of pro-inflammatory cytokine TNF- $\alpha$  and the production of NO. IL-10 production was significantly increased after extract treatment.

**Conclusion:** The results of the present study indicate the immunomodulatory effects of *A. catechu* extracts on humoral, cell mediated and non-specific immune functions.

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### 1. Introduction

The vertebrate immune system is a versatile defense system that is involved in protecting the host from invading pathogenic microorganisms. It also maintains a surveillance system that continuously monitors the integrity of host tissues [1]. The immune system influences pathophysiology of many infectious diseases. Such diseases can be alleviated by the modulation of immune responses. The two objectives of immunomodulation which is used to control diseases are stimulation of the immune system to develop immunity and

suppression of undesired immune responses. The traditional medicines play a pivotal role in stimulating and suppressing the host immune responses. Plant derived natural substances can serve as immunomodulators to control certain immune responses [2].

*Acacia catechu*, a deciduous tree of the *Fabaceae* family is indigenous in India, other Asian countries, and East Africa. It is commonly known as catechu, cachou and black cutch. This plant is widely used in Ayurveda for many diseases including skin diseases. Ayurveda uses bark and heartwood of this plant for various formulations. *Khadirarishta* is a famous Ayurvedic skin tonic prepared from *A. catechu*. *Khadira Sara*, the heartwood extract is used as ingredient in many medicines such as *Lavangadi Vati* [3]. “*Ercha*” is a traditional Chinese medicine prepared from the heartwood extract of catechu used in the treatment of cough, dysentery, skin ulcerations and lesions [4]. The gummy extract of the wood called black

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catechu, is used as an anodyne, astringent and bactericide. The heartwood extract is also used in asthma, bronchitis, colic, diarrhea, boils, skin afflictions, sores and stomatitis. The bark shows anti-helminthic, antipyretic and anti-inflammatory properties. It is used in the treatment of bronchitis, ulcers, psoriasis, anaemia and gum troubles. The immunomodulatory activity of aqueous extract of bark of *A. catechu* has been reported [5]. The sap of *A. catechu* is commonly used for the treatment of diarrhea and wounds in ruminants. In veterinary folk medicine, both the extracts of bark and heartwood are used for broken horn [6]. The decoction prepared from the heartwood of this plant is used for drinking purpose in southern part of India especially in Kerala.

The important chemical constituents isolated and characterized from *A. catechu* include catechin, rutin, isorhamnetin, epicatechin, kaempferol, 4-hydroxybenzoic acid, 3,4',7-trihydroxyl-3',5-dimethoxyflavone, quercetin, afzelechin, epiafzelechin, mesquitol, aromadendrin, ophioglonin, and phenol [7]. Catechins, rutin and isorhamnetin show antioxidant property by scavenging free radicals [8]. These compounds are largely present in the heartwood extract of *A. catechu* and may contribute to the biopotency of *A. catechu* [9]. In the present study, we report the immunomodulatory activity of heartwood extracts of *A. catechu* by analyzing their effect on phagocytosis, specific antibody and antibody producing cells in spleen, delayed type hypersensitivity (DTH), nitric oxide (NO) production, TNF- $\alpha$  and IL-10 production by LPS stimulated macrophages and splenocyte proliferation.

## 2. Materials and methods

### 2.1. Plant material and extraction

*A. catechu* was collected from Kannur District, Kerala, India in October, 2012. The specimen was authenticated by the taxonomist from Department of Botany, St. Thomas College, Pala, Kottayam, Kerala, India. Voucher specimens (Sunil MA 1504) have been deposited at herbarium of Department of Botany, St. Thomas College. Heartwood of the plant was shade dried, powdered and stored in sealed containers. The powder was defatted with hexane and then subjected to successive extraction using ethanol and water by Soxhlet extraction. The solvent was removed under reduced pressure and the extracts were stored at 4 °C, till used.

### 2.2. Animals

Swiss albino male mice (20–30 g) were obtained from Kerala Veterinary University, Thrissur, Kerala. They were maintained in animal house under standard conditions and fed with standard pellet diet and water *ad libitum*. The experiments were approved by Institutional Animal Ethics Committee (No: B21032014-08).

### 2.3. Sheep red blood cells (SRBCs) suspension

Fresh sheep blood from local slaughter house was collected in Alsever's solution (1:1 proportion) and was kept in the refrigerator. SRBC for immunization was prepared by centrifuging at 2000 rpm for 10 min and washing 4–5 times with physiological saline. The washed SRBCs were then suspended in buffered saline at desired concentration.

### 2.4. Treatment

The doses for the present study were fixed by Irwin test at 500, 1000 and 2000 mg/kg body weight concentrations [10]. The *A. catechu* water extract and ethanol extract were dissolved in phosphate-buffered saline (pH 7.2). For further assays, 1/10th–1/

20th of the dose at which behavioral alterations were observed was considered as safe dose [11]. The extracts did not produce any toxic effect up to 2 g/kg b. w. concentration. 100 and 200 mg/kg b. w. were fixed as the dose range for further studies. Six groups of Swiss albino mice (6 Nos/group) were used in the present study. Group I was control which received PBS. Group II were treated with dexamethasone (DMS) at a concentration of 1.25 mg/kg b. w. Animals of group III, IV, V, and VI received 100 and 200 mg/kg b. w. of the two extracts orally in volumes of 0.2 ml/day for 30 days.

### 2.5. Preparation of peritoneal mouse macrophages

One milliliter of 3% Brewer thioglycollate medium (Himedia, India) was injected intraperitoneally into mice as a stimulant to elicit peritoneal macrophages. Four days later, the peritoneal exudate was collected by peritoneal lavage with 10 ml of RPMI-1640 medium (Himedia, India). The exudate was centrifuged at  $400 \times g$ , 4° C for 10 min. The supernatant was discarded and cell pellet was resuspended in complete RPMI-1640 medium. Contaminating erythrocytes were lysed using erythrocyte lysis buffer. The cell number was determined by counting in a hemocytometer and the cell viability was tested by the trypan-blue dye exclusion technique [12].

### 2.6. Preparation of mouse splenocytes

Splenocytes were prepared according to the method previously described [13] with a slight modification. Briefly, mice were sacrificed and their spleens were removed aseptically. The cell suspension was prepared by means of flushing. After centrifugation at 1000 rpm at 37 °C for 10 min, erythrocytes were lysed by lysis buffer and the cell pellets were washed twice with RPMI-1640 medium. The cells were re-suspended in complete RPMI-1640 medium and the cell number was adjusted to desired concentration. The viability of splenocytes was determined by the trypan-blue dye exclusion technique as described earlier.

### 2.7. Hemagglutinating antibody (HA) titer

Hemagglutinin titer assay was performed using the procedure of Bin-Hafeez et al [14]. On the day of termination of the treatment, animals were immunized with 0.2 ml of 10% SRBCs intraperitoneally. On the fifth day after immunization, blood was collected from orbital plexus of each mouse and serum was separated. Serum was then incubated at 56 °C for 15 min to inactivate complement. Serial two fold dilutions of serum was made in 50  $\mu$ l of PBS (pH 7.2) in 96-well plates and mixed with 50  $\mu$ l of SRBC (1%) suspension in PBS. After mixing, plates were kept at room temperature for 2 h. The highest serum dilution which showed visible hemagglutination was considered as the antibody titer.

### 2.8. Plaque forming cell (PFC) assay

The effect of *A. catechu* extracts on antibody producing cells was studied by the method of Davis and Kuttan [15]. On the day of termination of the extract treatment, animals were immunized with  $2.5 \times 10^8$  SRBC, intraperitoneally. The animals were euthanized on the 5th day after immunization. Splenocytes were prepared as mentioned earlier. 50  $\mu$ l each of spleen cell suspension ( $1 \times 10^6$  cells/ml) and antigen (SRBC 7%) were mixed with 0.5 ml of molten agarose kept at 45 °C and spread on slides. After solidifying the agarose, rabbit serum (complement) was applied over the gel and incubated at 37 °C for 1 h. The number of plaque forming cells (PFC) was determined by Jerne's plaque assay [16].

### 2.9. Delayed type hypersensitivity (DTH)

The DTH response was determined using the method of Bin-Hafeez et al [17]. On the day of termination of the treatment, animals were immunized with SRBC ( $1 \times 10^9$  cells), subcutaneously. On the fifth day of immunization, all the animals were again challenged with  $1 \times 10^8$  cells in the left hind footpad. The right footpad injected with the same volume of normal saline served as a trauma control for non-specific swelling. Increase in footpad thickness was measured 24 h after the challenge using an engineering micrometer (Digimatic micrometer – Mitutoyo South Asia).

### 2.10. Nitroblue tetrazolium (NBT) reduction test

The NBT reduction assay was carried out as described by Rainard [18]. Macrophage suspension was seeded in each well of a 96-well plate and incubated for 2 h in 5% CO<sub>2</sub> humidified incubator; then non-adherent cells were removed by washing in RPMI-1640 medium. The remaining adherent cells ( $1 \times 10^6$  cells/well) were cultured in 100 µl RPMI-1640 medium with plant extracts dissolved in 0.1% dimethyl sulfoxide (DMSO) in PBS so that their final concentrations were 10 µg/ml, 25 µg/ml, 50 µg/ml, and 100 µg/ml and incubated for 24 h at 37 °C in 5% CO<sub>2</sub> humidified atmosphere. The 0.1% DMSO in PBS (without plant extract) was used as control. After incubation, 20 µl of the heat inactivated yeast (*Saccharomyces cerevisiae*) suspension ( $5 \times 10^7$  particles/ml) and 20 µl of NBT (Sigma, USA) solution in PBS (1.5 mg/ml) were added and the mixture was further incubated under the same conditions. After incubation for 60 min, the adherent macrophages were rinsed vigorously with RPMI medium and washed four times with 200 µl methanol. After air-drying, 120 µl of 2M KOH and 140 µl of DMSO were added. The absorbance was measured by a well reader (ThermoScientific, USA) at 570 nm and the percentage of NBT reduction was calculated by following equation:

$$\text{NBT reduction(\%)} = \frac{\text{OD sample} - \text{OD control}}{\text{OD control}} \times 100$$

### 2.11. Cellular lysosomal enzyme assay

Cellular lysosomal enzyme activity was measured by determining acid phosphatase in macrophages as described by Suzuki et al [19]. Macrophage suspension was seeded in each well of a 96-well plate and incubated in 5% CO<sub>2</sub> humidified incubator for 2 h; then non-adherent cells were removed by washing in RPMI-1640 medium. The remaining adherent cells ( $1 \times 10^6$  cells/well) were cultured in 100 µl RPMI-1640 medium with plant extracts dissolved in 0.1% dimethyl sulfoxide (DMSO) in PBS so that their final concentrations were 10 µg/ml, 25 µg/ml, 50 µg/ml, and 100 µg/ml and incubated for 24 h at 37 °C in 5% CO<sub>2</sub> humidified atmosphere. The 0.1% DMSO in PBS (without plant extract) was used as control. After incubation, the medium was removed by aspiration and 20 µl of 0.1% Triton X-100 (Himedia, India), 50 µl of 0.1 M citrate buffer (pH 5.0) and 100 µl of 10 mM p-nitrophenyl phosphate (p-NPP) (Himedia, India) solution were added into each well. The plate was further incubated for 30 min, 150 µl of 0.2M borate buffer was then added and the absorbance was measured at 405 nm. The lysosomal enzyme activity (%) was calculated by the following equation:

$$\text{Lysosomal enzyme activity(\%)} = \frac{\text{OD sample} - \text{OD control}}{\text{OD control}} \times 100$$

### 2.12. Nitric oxide (NO) assay

The peritoneal macrophages were seeded in 24-well plate and incubated for 2 h in 5% CO<sub>2</sub> humidified incubator; then non-adherent cells were washed out using RPMI-1640 medium. The remaining adherent cells ( $2 \times 10^6$  cells/well) were cultured in 1 ml RPMI complete medium containing 10% fetal calf serum (FCS) and incubated for 24 h with different concentrations of the plant extract (dissolved in 0.1% DMSO in PBS) ranging from 10 to 100 µg/ml both in the absence and presence of LPS (1 µg/ml). NO<sub>2</sub><sup>-</sup> concentration derived from NO in culture media was determined by the Griess method [20]. After incubation, 100 µl cell culture supernatant was added to 100 µl Griess reagent, and incubated at room temperature for 10 min in 96-well microplate. Absorbance was read at 540 nm in a microplate reader (ThermoScientific, USA). Sodium nitrite was diluted in the medium and used as a standard.

### 2.13. Assessment of cytokines secretion in macrophages

The macrophages were plated in 96-well plate and incubated for 2 h in 5% CO<sub>2</sub> humidified incubator; then non-adherent cells were removed by washing in RPMI-1640 medium. The remaining adherent cells ( $1 \times 10^6$  cells/well) were cultured at 37 °C in 5% CO<sub>2</sub> for 24 h in RPMI-1640 complete medium with or without LPS (1 µg/ml) in the presence of various concentrations (10–100 µg/ml) of the extracts. LPS treated cells were considered as positive control and cells without LPS treatment were considered as negative control. After 24 h, supernatants were collected by centrifugation at 2500 rpm for 20 min at 18 °C and assayed for IL-10 and TNF-α using ELISA kits (Biolegend, USA) according to the manufacturer's instructions.

### 2.14. Splenocyte proliferation assay

The proliferation assay of lymphocytes was carried out according to 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) method [21]. Briefly, 20 µl of various concentrations (10–100 µg/ml) of the plant extracts was added to 20 µl of spleen cell suspension ( $1 \times 10^6$  cells/ml) and 40 µl of RPMI-1640 medium in a 96-well plate. Proliferation of cells in the presence and absence of mitogens was investigated. The predetermined optimum dose of concanavalin-A (ConA), lipopolysaccharide (LPS) and phytohemagglutinin-M (PHA-M) at 5 µg/ml was used. After incubation at 37 °C in humidified 5% CO<sub>2</sub> for 48 h, 20 µl of MTT (Himedia, India) at concentration of 5 mg/ml in PBS and 40 µl of RPMI-1640 medium were added to the wells and incubated for additional 4 h under the same conditions. The culture medium was removed and 100 µl of 0.04 M HCl in isopropyl alcohol were added to lyse cells. Then, the solution was diluted by adding 100 µl of distilled water and the absorbance was measured at 570 nm. The percentage of proliferation was calculated by the following equation:

$$\text{Proliferation(\%)} = \frac{\text{OD sample} - \text{OD control}}{\text{OD control}} \times 100$$

### 2.15. Statistical analysis

All the values were expressed as mean ± SEM for six animals. Values for the *in vitro* assays were expressed as mean ± SEM for triplicate independent experiments. Statistical significance was analyzed using one-way ANOVA followed by Tukey's HSD using SPSS program (version 20). The differences were considered significant at  $p \leq 0.05$ .

### 3. Results

#### 3.1. Hemagglutinating antibody titer

The hemagglutinating antibody titer assay was used to assess the effect of extracts of *A. catechu* on humoral immune response. A dose dependent increase in the hemagglutinating antibody titer was observed with the extract treated groups compared to control groups. The animals treated with the ethanol extract of *A. catechu* (200 mg/kg b. w.) caused a significant rise in antibody titer (Table 1).

#### 3.2. Effect of *A. catechu* extracts on antibody producing cells

The treatment with *A. catechu* increased the number of antibody producing cells in spleen (Table 1). The maximum number of plaque forming cells was observed in ethanol extract treated group ( $535.67 \pm 1.69$  PFC/ $10^6$  spleen cells) at a dose of 200 mg/kg while the PBS treated animals had only  $330.83 \pm 3.22$  PFC/ $10^6$  spleen cells ( $p < 0.05$ ).

#### 3.3. Delayed type hypersensitivity (DTH) reaction

The effect of the extracts on T-cell mediated DTH reaction is shown in Table 1. The results showed that both the extracts at doses of 100 and 200 mg/kg significantly inhibited the foot paw edema compared to control groups.

#### 3.4. In vitro phagocytic activity assay of plant extracts in mouse macrophages

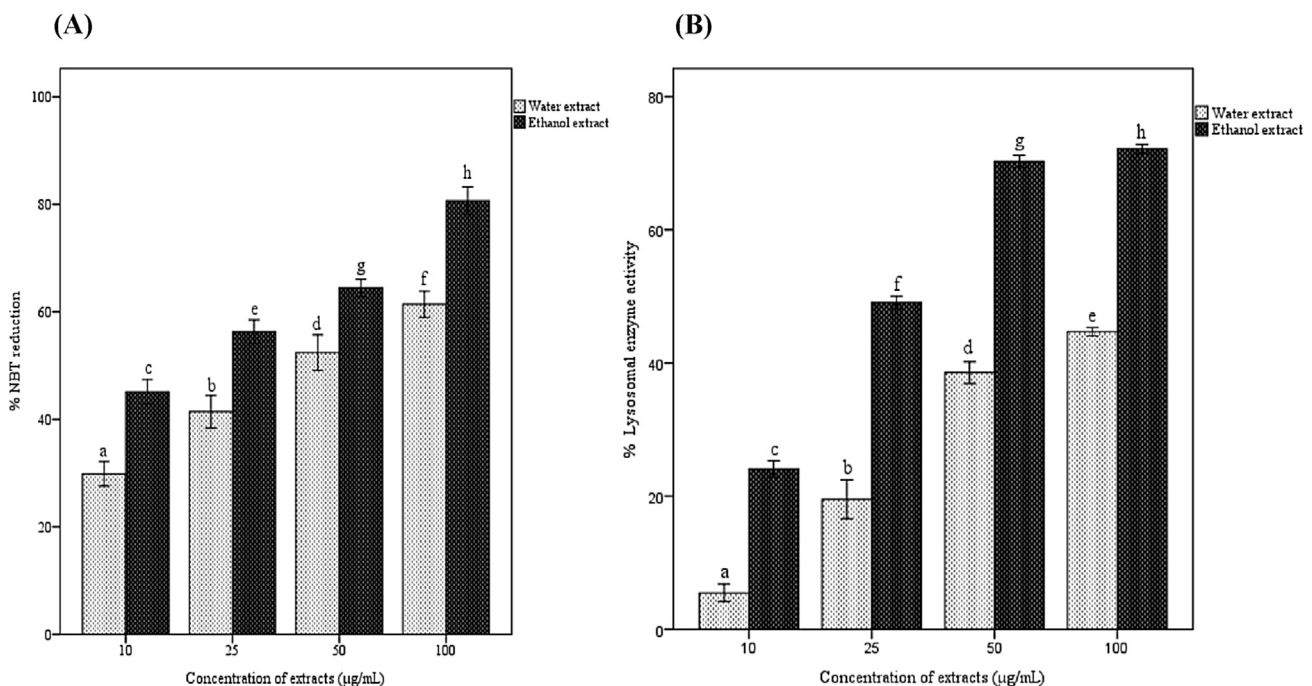
Effects of concentrations of the plant extracts on the reduction of NBT dye and lysosomal enzyme activity response in macrophages were demonstrated (Fig. 1A and B). Both water and ethanol extracts gave phagocytic modulation and a dose dependent response was observed. Phagocytic activity increased with increase in concentration of the extracts. Ethanol extract at a concentration of 100  $\mu$ g/ml showed maximum activity in NBT dye reduction (80%) and lysosomal enzyme activity (72%).

**Table 1**

Effect of *A. catechu* on HA titer, plaque forming cells and delayed type hypersensitivity.

Groups	HA titer	PFC/ $10^6$ spleen cells	DTH (footpad thickness in mm)
Control (PBS)	$14.67 \pm 1.33^a$	$330.83 \pm 3.22^b$	$2.25 \pm 0.07^a$
Water extract (100 mg/kg)	$29.33 \pm 2.67^a$	$356.67 \pm 1.71^c$	$1.05 \pm 0.02^b$
Water extract (200 mg/kg)	$58.67 \pm 5.33^b$	$370.50 \pm 1.33^d$	$0.9783 \pm 0.02^b$
Ethanol extract (100 mg/kg)	$58.67 \pm 5.33^b$	$429.83 \pm 1.51^e$	$0.2612 \pm 0.01^c$
Ethanol extract (200 mg/kg)	$117.33 \pm 10.67^c$	$535.67 \pm 1.69^f$	$0.1842 \pm 0.01^c$
Dexamethasone (1.25 mg/kg)	$7.33 \pm 0.67^a$	$204 \pm 2.62^b$	$0.1463 \pm 0.00^c$

Values represent mean  $\pm$  SEM for six animals. Values carrying same alphabet did not vary significantly from each other (Tukey's HSD;  $p \leq 0.05$ ).



**Fig. 1.** Effect of *A. catechu* on in vitro phagocytosis assay. Effect of *Acacia catechu* on (A) NBT reduction and (B) cellular lysosomal enzyme activity. Values represent mean  $\pm$  SEM of triplicate independent experiments; and values carrying same alphabet did not vary significantly from each other (Tukey's HSD;  $p \leq 0.05$ ).

### 3.5. Effect of *A. catechu* on NO production

NO production by peritoneal macrophages was inhibited by extract treatment in a dose dependent manner (Fig. 2). LPS stimulated macrophages produced NO up to  $49 \pm 0.72 \mu\text{M}$  whereas untreated macrophages produced only  $6.5 \pm 0.51 \mu\text{M}$ .

### 3.6. Effect of *A. catechu* on cytokine release

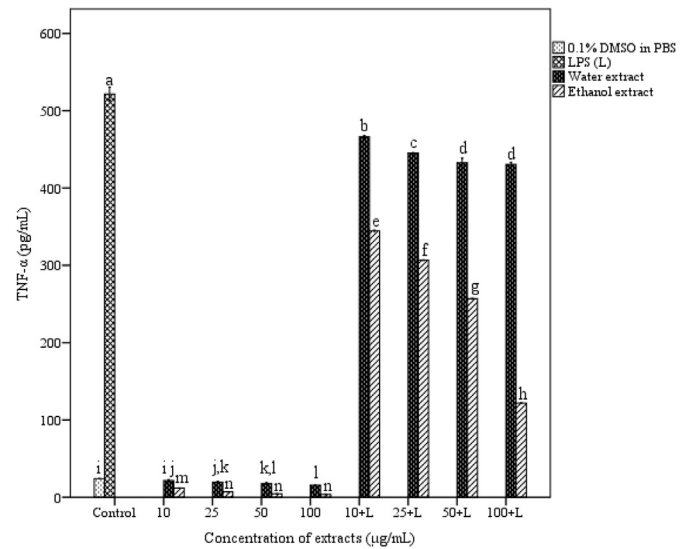
The effect of *A. catechu* on cytokine release in peritoneal macrophages is depicted in Fig. 3. TNF- $\alpha$  production by stimulated macrophages was significantly decreased in the presence of extracts. Ethanol extract showed more inhibition ( $121.78 \pm 0.01 \text{ pg/mL}$ ) compared to water extract ( $430.53 \pm 0.67 \text{ pg/mL}$ ) at a concentration of  $100 \mu\text{g/ml}$  ( $p < 0.05$ ) in the presence of LPS. Level of IL-10 was increased after extract treatment in a dose dependent manner (see Fig. 4).

### 3.7. Effect of extracts on mouse splenocyte proliferation

Effect of *A. catechu* on splenocyte proliferation with or without mitogens is shown in Fig. 5. All extracts showed cell proliferation stimulation with a dose response relationship. The ethanol extract gave the maximum proliferation enhancement both in the presence and absence of mitogens. In the absence of mitogen, ethanol extract at  $100 \mu\text{g/ml}$  enhanced proliferation by 111% ( $p < 0.05$ ) compared to the control. In the presence of LPS, the ethanol extract stimulated splenocyte proliferation by 157% ( $p < 0.05$ ) at  $100 \mu\text{g/ml}$ .

## 4. Discussion

Plants are good sources of biologically active natural products. Phytomedicines are used as health supplements or alternative therapies for treatment of diseases. Plant derived compounds have been used as the starting material for many semi-synthetic drugs. Herbal agents are often considered as safer and are of lower cost than conventional chemotherapeutics [22]. Immunomodulatory phytocompounds from herbal agents activate host defense

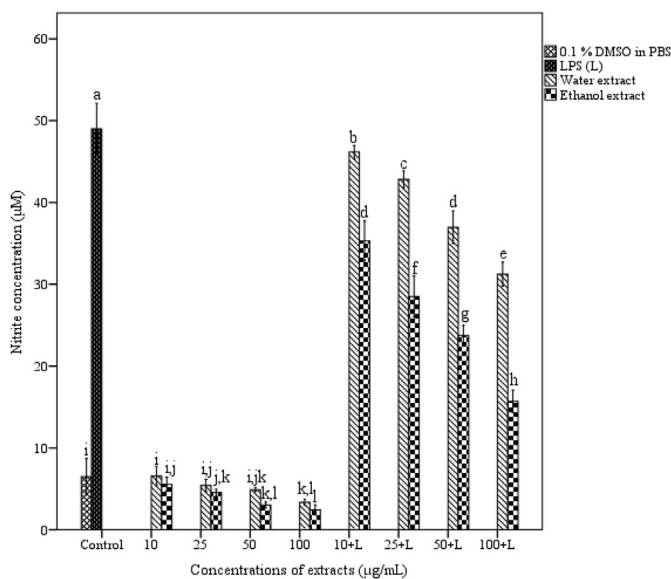


**Fig. 3.** Effect of *A. catechu* heartwood extracts on secretion of TNF- $\alpha$  in peritoneal macrophages. Values represent mean  $\pm$  SEM of triplicate independent experiments; and values carrying same alphabet did not vary significantly from each other (Tukey's HSD;  $p \leq 0.05$ ).

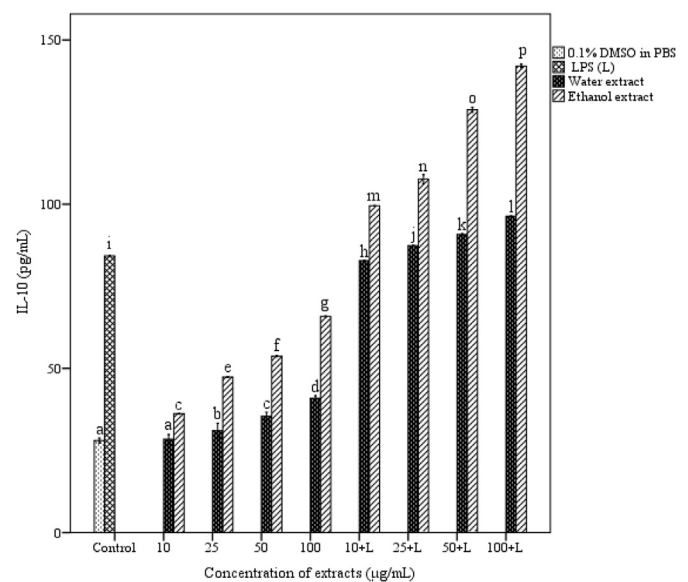
mechanism and can provide an alternative therapy to conventional chemotherapy [23].

In the present study, treatment with *A. catechu* heartwood extracts enhanced the antibody titer. This indicates the stimulatory effect of the extracts on humoral response to SRBC [24]. Spleen plays an important role in developing immune responses to antigens in the bloodstream. Plaque forming cell assay was used to determine the number of antibody producing cells in the spleen. The significant enhancement of antibody producing cells in the spleen also indicates an activated humoral immune response.

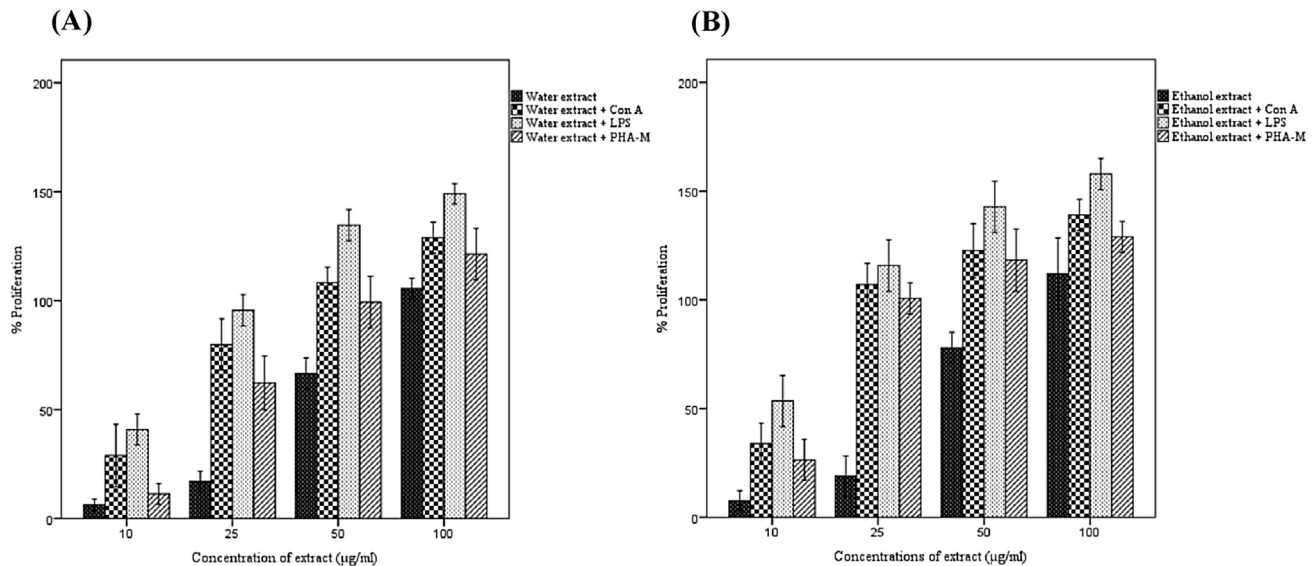
DTH reaction directly correlates with cell-mediated immunity mediated by sensitized T-lymphocyte [25]. DTH response was



**Fig. 2.** Effect of *A. catechu* on NO production. Values represent mean  $\pm$  SEM of triplicate independent experiments; and values carrying same alphabet did not vary significantly from each other (Tukey's HSD;  $p \leq 0.05$ ).



**Fig. 4.** Effect of *A. catechu* heartwood extracts on secretion of IL-10 in peritoneal macrophages. Values represent mean  $\pm$  SEM of triplicate independent experiments; and values carrying same alphabet did not vary significantly from each other (Tukey's HSD;  $p \leq 0.05$ ).



**Fig. 5.** Effect of *A. catechu* heartwood extracts on *in vitro* proliferation response of mouse splenocytes. (A) Effect of water extract on splenocyte proliferation. (B) Effect of ethanol extract on splenocyte proliferation; values represents mean  $\pm$  SEM of triplicate independent experiments comparing to the control; and values carrying same alphabet did not vary significantly from each other (Tukey's HSD;  $p \leq 0.05$ ).

significantly decreased after extracts treatment, indicating that immune response to SRBC is mainly due to the presence of high titer of antibodies and not due to generalized stimulation of immune responses involving T-cells. Reduction of NBT in NBT assay represents the activity of NADPH oxidase enzyme reflecting the stimulation of phagocytes [18]. NADPH oxidase enzyme plays an important role in the generation of reactive oxygen species such as superoxide radical which are involved in intracellular killing of pathogens by phagocytes. Yellow coloured, nitroblue tetrazolium undergo reduction by  $O_2^-$  and form blue NBT formazan. The cellular lysosomal enzyme assay is used to determine acid phosphatase secretion in macrophages. The membrane associated acid phosphatase of treated macrophages transforms p-nitrophenyl phosphate (p-NPP) to a coloured compound, indicating the activity of extract on degranulation of macrophages [19]. In the present study, lysosomal enzyme activity was significantly increased after extracts treatment. This also represents a stimulatory effect on phagocytes.

NO is an important inflammatory mediator. Small amount of NO is required for many physiological functions. Over production of NO may lead to inflammatory diseases. NO production was significantly decreased both in LPS stimulated and unstimulated macrophages treated with extracts. This indicates the anti-inflammatory effect of the extracts. The anti-inflammatory property of *A. catechu* was further demonstrated by analyzing the level of TNF- $\alpha$  and IL-10 secretion in macrophages. TNF- $\alpha$ , a strong mediator of inflammatory response, is secreted by monocytes and macrophages. It is involved in many of the clinical dilemma associated with autoimmune disorders such as rheumatoid arthritis, inflammatory bowel diseases and psoriasis. *A. catechu* inhibited the production of TNF- $\alpha$ . IL-10 plays an important role in immunoregulation and inflammation. IL-10 enhances proliferation of B cells, thymocytes and mast cells and can suppress the secretion of proinflammatory cytokines from macrophages. It can block the activity of NF- $\kappa$ B, a major transcription factor that regulates genes involved in inflammation. In this study, IL-10 level was increased with extract treatment. The extracts stimulated splenocyte proliferation both in the presence and absence of mitogens. Maximum proliferative

effect was shown in the presence of LPS which is involved in T cell independent B cell proliferation.

The anti-inflammatory activity of *A. catechu* has been demonstrated by reducing the production of proinflammatory eicosanoids [26]. *A. catechu* heartwood is a rich source of catechin and epicatechin. Catechin and epicatechin are frequent components of traditional herbal remedies. Catechin is a plant flavonoid which possesses antioxidant properties. Presence of catechin may be the reason for its observed immunomodulatory activity.

## 5. Conclusion

The traditional knowledge on the medicinal uses of *A. catechu* has been scientifically validated in this study. The effect of the plant on enhancement of HA titers, PFC assay as well as its effect on DTH reaction and peritoneal macrophages indicate that it has appreciable immunomodulatory effect. The increased antibody titer indicates that daily consumption of *A. catechu* decoction may provide immunity against infections in general. A detailed study on the level of individual immunoglobulin classes is important to confirm the assumption. As it is not possible at this point to elucidate all the effective immunomodulatory constituents of *A. catechu*, further detailed studies in this regard will be helpful.

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## Conflict of interest

None.

## Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.jaim.2017.10.010>.

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