Immunomodulatory Activity of Alcohol Extract of *Terminalia chebula* Retz Combretaceae

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Abstract

**Purpose:** To investigate the immunomodulatory activity of the alcohol extract of *Terminalia chebula* Retz (Combretaceae) dried ripe fruits at the cellular level.

**Methods:** For antioxidant study, the liver mitochondria were separated and used for the estimation of enzymes catalase (CAT) and superoxide dismutase (SOD) - as well as lipid peroxidation (LPO) and reduced glutathione (GSH); Melatonin secretion was characterized using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) while spleen lymphocyte proliferation assay was performed by measuring optical density at 570 nm using ELISA reader. The cytokines, namely, IL-2, IL-10 and TNF-α expression in spleen cells, were determined by real time polymerase chain reaction (RT-PCR)

**Results:** *Terminalia chebula* extract (100 mg/kg/p.o.) increased the level of liver mitochondrial enzymes CAT and SO as well as GSH but decreased the level of LPO in the liver when compared to the vehicle, sheep red blood cells (SRBC) and cyclophosphamide-treated groups. Secretion of melatonin by pineal gland was enhanced by *T. chebula* treatment. The extract also increased spleen lymphocyte proliferation. Based on RT-PCR analysis, the expression of cytokines, viz. IL-2, IL-10 and TNF-α, was more in *T. chebula*-treated than in vehicle- and cyclophosphamide- treated groups.

**Conclusion:** This study confirms the immunomodulatory activity of ripe *T. chebula* fruits as evidenced by increase in the concentration of antioxidant enzymes, GSH, T and B cells, the proliferation of which play important roles in immunity. This phenomenon also enhances the concentration of melatonin in pineal gland as well as the levels of cytokines, such as IL-2,IL-10 and TNF-α, which play important roles in immunity.

**Keywords:** *Terminalia chebula*, Antioxidants, SDS-PAGE, Lymphocytes proliferation, Real-time PCR, Immunity

Received: 2 January 2011

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Trop J Pharm Res, October 2011;10(5):567
INTRODUCTION

Herbal medicine has become an integral part of standard healthcare, based on a combination of time-honoured traditional usage and ongoing scientific research. Rising interest in medicinal herbs has increased scientific scrutiny of their therapeutic potentials and safety. Some of the medicinal plants are believed to enhance the natural resistance of the body to infections [1].

Immunostimulation and immunosuppression both need to be tackled in order to regulate normal immunological functioning. Therefore, stimulatory or suppressive agents which possess activity to normalize or modulate pathophysiological processes are called immunomodulatory agents. Among suppressive synthetic substances, cyclophosphamide has been extensively studied. However, the major drawback of this drug is myelosuppression, which is undesirable [2]. Moreover, natural adjuvants, synthetic agents and antibody reagents are used as immunomodulatory agents. Nevertheless, there are major limitations to the general use of these agents such as increased risk of infection and generalized effect throughout the immune system.

There is a rise in the usage of herbal plants to treat diseases of the immune system over the last century. Besides, compared to synthetic drugs, herbal drugs are frequently considered to be less toxic and with fewer side effects. Therefore, the search for more effective and safer agents that exert immunomodulatory activity has intensified across the world [3].

In recent years, immunostimulatory activity has been reported in a number of Ayurvedic plants such as Tinospora cordifolia, Allium sativum, Andrographis paniculata, Picrorrhiza kurroa [4] and Terminalia chebula. T. chebula has antibacterial, antifungal, antiviral, antimutagenic/anticarcinogenic, hepatoprotective, cardioprotective and radioprotective activities [5]. In spite of its many therapeutic effects, to the best of our knowledge, no data are available on the immunomodulatory effect of the dry ripe fruits of Terminalia chebula at the cellular level. Thus, the objective of the present study was to assess the immunomodulatory potential of the alcohol extract of the dry ripe fruit of this plant at the cellular and molecular levels using Wistar male rats.

EXPERIMENTAL

Plant material and preparation of extracts

The ripe fruits of Terminalia chebula were identified by Dr GC Joshi (taxonomist), Regional Research Institute, Ranikhet, Uttarakhand, India. A voucher specimen (no. PHG/H/2132) has been preserved in the herbarium at the Pharmacognosy laboratory, College of Pharmacy, IFTM Moradabad for future reference. The fruits of T. chebula were shade-dried and coarsely powdered, 335 g of which extracted with 1.5 L of 70 % ethanol for 32 h using a Soxhlet apparatus. The extract was concentrated to dryness under reduced pressure by rotary evaporator at 45 °C yielded 16 g of dry extract which was then preserved in a calcium chloride dessicator pending further studies.

Animals

Male Wistar rats weighing 150 - 180 g were procured from Laboratory Animals Resources, Division of Animal Genetics, Indian Veterinary Research Institute (IVRI), Izatnager, Bairelly, India and acclimatized to laboratory conditions in the animal house of IFTM, Moradabad at room temperature with a 12h/12h light/dark cycle and relative humidity of 60 ± 5 %. They had free access to standard pellet diet and water. The institutional animal ethical committee reviewed and approved (reg no. 837/ac/04) the protocol of the animal studies prior to commencement of the experiment. All the rats were treated in accordance with the National Institutes of Health’s Guidelines for the Care and Use of Laboratory.
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Preparation of sheep red blood cells (SRBC)

Blood from a healthy sheep, was collected from a local abattoir, mixed thoroughly with sterile Alsever’s solution (1:1) and centrifuged at 3000 rpm for 5 min. The supernatant was discarded, and the SRBC pellets were washed thrice with sterile phosphate buffered saline (pH 7.2), re-suspended in phosphate buffered saline (pH 7.2). Total SRBC was counted using Neubauer chamber. SRBC (1x10^8 cells, 0.5ml) was injected intraperitoneally to produce sensitization of the rats [6].

Treatment of animals

The rats were divided into four groups of six rats each. Group I was negative control and treated with 2 ml of 1 % gum acacia solution in distilled water. Group II was positive control: where the rats (first sensitized by administering 1 x 10^8 SRBC, i.p.) were treated with 1 % gum acacia solution orally. Group III rats were treated with cyclophosphamide (100 mg/kg, p. o). Group IV was sensitized rats treated with the extract (100 mg/kg, p.o.). The foregoing treatments were administered to Groups II and IV 4 days prior to sensitization (days -3, -2, -1, 0) where applicable, and for 7 days after sensitization (days +1, +2, +3, +4, +5, +6, +7)

Assessment of immunological activity

Immunostimulatory activity was examined using in vivo and in vitro immunological tests which are described below Six hours after the last dose, the animals were sacrificed, the liver, pineal gland and spleen removed and their biochemical parameters determined as described in the following subsections.

To isolate the mitochondria, 1 g of liver tissue was weighed, homogenized with 5 ml 0.35M sucrose buffer (pH 7.0) at 4 °C and centrifuged at 10,000 g for 5 min. The supernatant was discarded and the resultant pellet, which contained mitochondria, was suspended in a mixture of 1 ml of 10mM Tris-HCl (pH 7.4) solution and 0.2 ml of 1mM EDTA; the volume was made up to 2 ml with 0.25M sucrose solution [7]. This solution was used for the determination of various mitochondrial antioxidant enzymes.

Determination of lipid peroxidation (LPO) in homogenized liver tissue

After sacrificing the animals, the liver was isolated, weighed, washed with chilled ice-cold sterile 0.9 % NaCl solution. Tissue homogenate was prepared in a ratio of 1 g of wet tissue to 9 ml of 1.15 % KCl using a Teflon Potter-Elvehjem homogenizer [7].

The tissue homogenate (0.2 ml) was added to a mixture of 0.2 ml of 1% sodium dodecyl sulfate (SDS), 1.5 ml of 20 % acetic acid solution adjusted to pH 3.5 with NaOH, and 1.5 ml of 0.8 % aqueous solution of thiobarbituric acid. The mixture was made up to 4 ml with distilled water, and then heated in an oil bath at 95 °C for 60 min using a glass ball as a condenser. After cooling the mixture in tap water, 1 ml of distilled water, and 5 ml of mixture of n-butanol and pyridine (15:1, v/v) were added and shaken vigorously. After centrifuging at 4000 rpm for 10 min, the organic layer was taken and the absorbance measured at 532nm (model UV-1240m spectrophotometer, Shimadzu, Japan). 1, 1, 3, 3- tetramethoxypropane (TMP) was used as standard, and the content of LPO was expressed as nmol of malondialdehyde (MDA) [7].

Reduced glutathione (GSH)

Mitochondrial GSH was estimated by adding 0.2 ml of mitochondrial enzyme solution to 1.8 ml distilled water followed by the addition of 3 ml precipitating mixture (0.0501g metabisulfatic acid, 0.006 g EDTA and 0.9 g NaCl in 3ml distilled water). It was centrifuged at 5000 g for 5 min, and 1 ml of the supernatant was collected, mixed first with 1.5 ml of phosphate buffer solution (pH 7.6) and then with 0.5 ml of 5,5'-dithio-bis -2-

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nitrobenzoic acid (DTNB) reagent. The optical density at 412 nm was measured spectrophotometrically and expressed in µMol/g Hb [7].

**Catalase (CAT)**

Mitochondrial enzyme solution (0.2 ml) was added to a cuvette containing 2 ml of phosphate buffer (pH 7) and 1 ml of 30mM H₂O₂. Catalase activity was measured spectrophotometrically (UV-1240 Shimadzu, Japan) at 412 nm for 1 min. The molar extinction coefficient of H₂O₂, 43.6 M cm⁻¹, was used to determine catalase activity. One unit of activity is equal to one millimole of H₂O₂ degraded per minute and is expressed as units/mg of protein [7].

**Superoxide dismutase (SOD)**

Mitochondrial enzyme solution (50µL) was added to a mixture of 0.5 ml of 75 mM Tris-HCl buffer (pH 8.2), 1 ml of 30mM EDTA and 1ml of 2mM pyrogallol. Absorbance was recorded at 420 nm for 3 min using a spectrophotometer. One unit of enzyme activity was calculated as 50 % inhibition of the rate of auto-oxidation of pyrogallol as determined by a change in absorbance/min at 420 nm. The activity of SOD was expressed as units/mg protein [7].

**Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)**

Protein fractions were isolated from the pineal glands of animals of each group and subjected to SDS-PAGE analysis for the detection of melatonin secretion. For this, the resolving gel mixture and stacking gel mixture used were 12 % resolving gel and 5 % stacking gel, respectively. The gels were stained using Coomassie Brilliant Blue R-250 solution for 4 h and then kept in destaining solution for 8 h until the background was colourless. Finally, after taking their photographs, the gels were stored in distilled water containing 20 % glycerol pending further study [8].

**Lymphocyte proliferation assay**

Splenocyte single cell suspension was prepared by up-downing 4 ml RPMI-1640 in spleen and after omitting RBCs using 0.75 % NH₄Cl in Tris buffer (0.02 %, pH 7.2) Six milliters of the buffer was added to 2 ml of the cell suspension and centrifuged at 1000 g for 2 min). The concentration was adjusted to 2 x 10⁶ cells/ml in RPMI-1640 supplemented with 10 % fetal calf serum, 2 mM L-glutamine, 25 mM (4-(2-hydroxyethyl)-1-piperazineethane-sulfonic acid) (HEPES). The diluted cell suspension (100 µl) was dispensed into a 96-well flat bottom culture plate. Mitogen phytohemmaggulatin A (PHA) was added at a final concentration of 5 µg/ml to each well and the volume adjusted to 0.2 ml. Following incubation for 72 h at 37 °C and 5 % CO₂ humid atmospheric air, cell proliferation was determined by MTT assay method. A solution (10 %) of 3-(4, 5 dimethyl-2-thiazoly) 2, 5-diphenyl-2H-tetrazolium (MTT, 5 mg/ml) was added to each well and the plate incubated at 37 °C in a CO₂ humid atmosphere for 4 h The blue formazan precipitate formed was dissolved in acidic isopropanol (0.1M HCl in absolute isopropanol) and its optical density measured at 570 nm using ELISA Reader. Stimulation index (SI) was calculated as in Eq 1 [9].

\[
SI = \frac{ODSC}{ODUC} \quad (1)
\]

where ODSC is the optical density of the stimulated cells and ODUC is the optical density of unstimulated cells

**Real Time PCR (RT--PCR) for determination of IL-2, IL-10 and TNF-α expression**

Spleen cell suspension was prepared and used for the determination of cytokines IL-2, IL-10 and TNF-α. The sequences of primers used in RT-PCR are given in Table 1.

**RNA isolation**

Trizol LS reagent (Invitrogen, cat no. 10296-010) Foster City, California, USA, was used
for RNA isolation using the method prescribed by the manufacturer. Briefly, spleen cells were washed with PBS, lysed by adding Trizol LS reagent to them and mixed by repeated pipetting. The organic and aqueous phases were separated with the aid of chloroform (0.2 ml) and centrifugation at 12000 g for 15 min. at 4 °C. Total RNA in the aqueous phase was precipitated with 0.5 ml isopropanol alcohol, washed in 75 % ethanol (1ml), air-dried and diluted in 50 µl nuclease-free water.

Table 1: The sequences of primers used in RT-PCR

| Primer   | Sequence (5’ to 3’)                           | Length (bases) |
|----------|-----------------------------------------------|----------------|
| IL-10Rat-F | ACCAGCTGGACAACATCTGCTGA                      | 24             |
| IL-10Rat-R | CCTTGATTCTGCGCATGGTTCT                     | 24             |
| IL-2Rat-F  | CTGCAGCGTGTGATGGATTTGACT                    | 24             |
| IL-2Rat-R  | TCGCTGGCTATCGATTTGACAT                     | 24             |
| BactRat-F  | TGAGAGGAATCGTGGCTGACAT                      | 24             |
| BactRat-R  | ACCGCTTATTGCGATTTGACAT                      | 24             |
| TNF Rat-F  | CTGGCAATGGCATGGATCTCAA                     | 24             |
| TNF Rat-R  | ATGAATGCGCAATCGGCTGACGG                   | 24             |
| RT-BGF    | CATGTATGGATGGCGCTGACCAA                    | 24             |
| RT-BGR    | TAAATGCCCTCCAGTGGCAAAAGT                  | 24             |

Complementary DNA (cDNA) preparation

Revert Aid M-MuLV Reverse Transcriptase (cat no. EP0441, Fermentas, Lithuania, Europe) was used for this purpose. About 100 ng RNA (accurately weighed) and 0.5 µg oligo dT primer (Invitrogen, cat no. 18418-012) were mixed and snap-chilled. To this, was added 4 µl of 5x reaction buffer, 20 U of RNase inhibitor, 2 µl of 10 mM dNTP and 200 U of Revert Aid M-MuLV Reverse Transcriptase. The final reaction volume was kept at 20 µl and incubated at 42 °C for 1 h. The reaction was then stopped by heating the mixture at 70 °C for 10 min. The resulting cDNA was divided into aliquots of suitable size and stored at -20 °C [10].

Real-time PCR studies

Brilliant SYBR Green QPCR Core Reagent Kit (Stratagene, Santa Clara, CA -USA cat no. 600546) was used for this purpose. One microliter of cDNA was added to 11 µl of nuclease free water, 2.5 µl of 10x core PCR buffer, 1.25 µl 50 mM MgCl₂ solution, 1 µl 20 mM dNTP mix, 1 µl (12.5 pM/µl) of forward primer, 1 µl (12.5 pM/µl) of reverse primer, 4 µl of 50 % glycerol solution, 0.75 µl of 100 % dimethyl sulfoxide (DMSO), 1.25 µl of 1:3000 diluted SYBR Green I dye and 0.25 µl (1.25 U) of SureStart Taq DNA polymerase. Real-time PCR was carried out in Mx3000p (Stratagene Santa Clara, CA -USA). The thermal profile used for this is as follows: 95 °C for 10 min then 40 cycles of 95 °C for 30 s, 64 °C for 30 s and 72 °C for 30 s with fluorescence recording at the end of each cycle, followed by denaturation of products from 55 to 95 °C with fluorescence recording throughout the step. Fold changes in target transcript levels were determined using the relationship: 

\[ E_{\text{target}} = E_{\text{ref}} = 2 \]

Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene was used as reference [10].

Statistical analysis

The data were expressed as mean ± standard deviation (SD) and statistical evaluation of the data was done using Prism software (version 5.00 GraphPad Software, Inc.USA). Data were tested for statistical significance using Student’s t-test, and differences were considered significant at \( p < 0.05 \).

RESULTS

Rat liver oxidative stress parameters

Table 2 shows increased lipid peroxidation level in the liver of SRBC-treated group (98.38 ± 1.14 nmol MDA/g Hb) compared to vehicle treated rat (83.85 ± 2.12 nmol MDA/g Hb). Furthermore, cyclophosphamide-treated
Table 2: Effect of *T. chebula* extract on rat liver oxidative stress parameters

| Treatment group | LPO (nmol MDA/g Hb) | GSH (µMol/g Hb) | SOD (units/mg protein) | Catalase (units/mg protein) |
|-----------------|----------------------|-----------------|------------------------|---------------------------|
| Vehicle         | 83.85±2.12           | 4.22±0.21       | 22.22±0.10             | 271.31±3.2                |
| SRBC-sensitized | 98.38±1.14           | 2.13±0.18       | 19.48±0.16             | 252.22±2.5                |
| CP              | 90.42±2.13           | 2.31±0.38       | 20.38±0.18             | 256.29±3.8                |
| *T. chebula*    | 68.01±1.16*          | 4.50±0.81*      | 25.36±0.21*            | 273.32±3.2*               |

*P < 0.05 compared with SRBC-sensitized and cyclophosphamide (CP)-treated groups.

Rats showed an increase (90.42 ± 2.13 nmol MDA/g Hb) in lipid peroxidation level in the liver but after *T. chebula* extract treatment, the elevated lipid peroxidation decreased to 68.01 ± 1.16 nmol MDA/g Hb.

The level of reduced glutathione, superoxide dismutase while catalase decreased on SRBC sensitization but was increased on treatment with *T. chebula* extract. The levels of reduced glutathione, superoxide dismutase and catalase on SRBC sensitization and extract treatment were 2.13 ± 0.18 and 4.50 ± 0.81 µMol/g Hb; 19.48 ± 0.16 and 25.36±0.21 units/mg protein; and 252.22 ± 2.50 and 273.32 ± 3.2 units/mg protein, respectively. Cyclophosphamide produced immunosuppression by increasing lipid peroxidation and decreasing the levels of reduced glutathione, superoxide dismutase and catalase (*p* < 0.05) when compared to the control group.

Separation of pineal proteins

A thick band at 248 kDa appeared in the group treated with the extract (lane 2) indicates a higher secretion of melatonin (Mol. Wt. 248 kDa). In the SRBC-sensitized group, the expression of proteins was less (lane 4) than the control group (lane 3). The immunosuppressant, cyclophosphamide, showed the least expression of proteins compared to the other groups (lane 5). The increased expression of melatonin in lane 2 confirms the immunomodulatory activity of the extract, as shown in Fig 1.

Figure 1: SDS-PAGE of rat pineal gland. **Lane 1:** Indicates the protein marker of the standard; **Lane 2:** *T. chebula* extract treatment shows thick band of protein expression (248kDa) indicating the increased secretion of melatonin via pineal gland due to immunomodulatory action; **Lane 3:** Control group; **Lane 4:** SRBC-sensitized group; **Lane 5:** Immunosupression action of cyclophosphamide.

Lymphocyte proliferation

Table 3 shows that administration of the extract increased spleen lymphocytes proliferation (1.332 ± 0.214) compared to the control (0.787 ± 0.256) and cyclophosphamide-treated rats (0.832 ± 0.471). Phytohaemagglutinin produced proliferation of lymphocytes in all the groups.

Table 3: Proliferation of rat spleen lymphocytes in response to antigen from the extract

| Treatment          | Stimulation index | Phytohaemagglutinin stimulated |
|--------------------|-------------------|-------------------------------|
| Vehicle            | 0.787±0.256       | 1.222±0.841                   |
| CP                 | 0.832±0.471       | 0.936±0.412                   |
| *T. chebula*       | 1.332±0.214*      | 1.233±0.514*                  |

*P < 0.05 compared with control and cyclophosphamide (CP)-treated groups.
IL-2, IL-10 and TNF-α gene expression in rat spleen mRNA

Measuring and assessing cytokine profile provides a useful method for accurate study of the cytokines such as IL-2, IL-10 and TNF-α. Table 4 shows that extract treatment increased the levels of IL-2, IL-10 and TNF-α mRNA several fold (7.46-, 73.52- and 6.23-fold, respectively) compared to control, cyclophosphamide and SRBC-sensitized groups. Increased expression in mRNA levels by the extract confirms its immunostimulant action.

Table 4: Effect of different treatments on IL-2, IL-10 and TNF-α gene expression

| Treatment          | Fold-change in IL-2, IL-10 and TNF-α mRNA level |
|--------------------|-----------------------------------------------|
|                    | IL-2  | IL-10 | TNF-α |
| Vehicle (Control)  | 1.00  | 1.00  | 1.00  |
| CP*                | 6.41  | 8.63  | -1.78 |
| T. chebula         | 7.46  | 73.52 | 6.23  |
| SRBC sensitized    | -2.87 | 22.63 | 1.18  |

*CP = cyclophosphamide

DISCUSSION

Oxidation of membrane lipid molecules causes damage resulting in the development of several physiological and pathological disorders. Inhibition of lipid peroxidation is the best way to avoid these disorders in the body [11]. The present study shows that T. chebula alcohol extract inhibited lipid peroxidation compared to treatment with SRBC and cyclophosphamide.

Glutathione is the major endogenous antioxidant produced by cells. It participates directly in the neutralization of free radicals and reactive oxygen compounds, as well as maintenance of exogenous antioxidants, such as vitamins C and E, in their reduced (active) forms. Through direct conjugation, it detoxifies many xenobiotics (foreign compounds) and carcinogens, both organic and inorganic [12]. It is essential for the immune system to exert its full potential, viz, modulating antigen presentation to lymphocytes, thereby influencing cytokine production; enhancing proliferation of lymphocytes and hence increasing the magnitude of response; and increasing the killing activity of cytotoxic T cells and natural killer cells, and regulating apoptosis, thereby maintaining control of the immune response[13]. 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 [14].

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 H₂O₂, thereby protecting the cell against free radical-induced damage [12].

Catalase is an antioxidant enzyme that helps in neutralizing the toxic effect of H₂O₂. Hydrogen peroxide is not reactive enough to cause a chain of lipid peroxidation reactions, but its combination with superoxide radical produces hydroxyl radicals that are highly reactive and thus initiate lipid oxidation reaction [15]. This lipid peroxidation damages the cell membrane resulting in the development of several physiological and pathological disorders. Catalase prevents this by conversion of hydrogen peroxide to water and non-reactive oxygen species, thereby preventing the generation of hydroxyl radical and protecting the cell from oxidative damage [16]. Our studies showed that there was distinct increase in the levels of glutathione, superoxide dismutase and catalase following treatment with T. chebula as alcohol extract compared to treatment with SRBC and cyclophosphamide. Therefore, the extract has both antioxidant as well and
immunomodulatory activities, and is thus capable of protecting cells from oxidative damage.

Melatonin plays an important role as an immunomodulator, as well as performing other physiological functions. It has been reported to be an integral part of the immune system, and exerts direct and/or indirect stimulatory effect on both cellular and humoral immunity [17]. The present study showed that treatment with *T. chebula* caused the expression of a thick band of protein, indicating increased production of melatonin. Hence, *T. chebula* extract exhibits immunostimulant action by enhancing melatonin secretion in the pineal gland.

Earlier workers have shown that drugs having immunomodulatory activity show enhanced proliferation of splenocytes [18]. In the present study, the extract significantly enhanced the proliferation of splenocytes. Proliferation of lymphocytes indicates increase in the number of β and T cells, which release cytokines and growth factors that regulate other immune cells and secretion of antibodies in the blood [19].

Cytokines are released by living cells of the host in a highly regulated fashion to regulate cell functions via specific receptors which participate in the control of all immunologically relevant events, whether they concern the activation, differentiation, maturation, proliferation, apoptosis, or acquisition of effector functions. Cytokines influence the quantitative, as well as the qualitative outcome of immune response [20]. In our study, administration of *T. chebula* extract to rats showed significant immunomodulatory activities as reflected by marked changes in IL-2, IL-10 and TNF-α mRNA levels. IL-2 shows immunomodulatory activities through T and β lymphocytes proliferation, natural killer cell activation [20-21] and increased immunoglobulin G production whereas IL-10 promotes elevation of Th2 cells [20-22] and TNF-α modulates cytokine gene expression [20]. Thus, the immunomodulatory activity of *T. chebula* may be by one or more of foregoing mechanism(s).

**CONCLUSION**

The immunomodulatory activity of *T. chebula* may be by inhibition of lipid peroxidation and increased levels of antioxidant enzymes catalase and superoxide dismutase; increased melatonin secretion by pineal gland which play a role in immunomodulatory action by exerting direct and/or indirect stimulatory effect on both cellular and humoral immunity; and proliferation of lymphocytes as indicated by the increase in the number of β and T cells which release cytokines and growth factors that regulate other immune cells and secretion of antibodies in the blood. Other mechanisms such as increased levels of cytokines IL-2, IL-10 and TNF-α which play important role in immunomodulatory actions such as T and B lymphocyte proliferation, natural killer cell activation, elevation of Th2 cells and modulation of cytokine gene expression also seem to be in operation. However, further investigations are required to isolate the active constituents responsible for these activities and to elucidate in detail the cellular and molecular mechanisms of action.

**ACKNOWLEDGEMENT**

The author is thankful to Drs Arvind Sonawane, Division of Genetics, Abhijit Pawdey, Division of Surgery; Sameer Shrivastava and Sonal Shrivastava Peptide Synthesis and Protein Engineering Lab, Division of Animal Biotechnology, for assistance in performing RT-PCR, lymphocyte proliferation assay at Indian Veterinary Research Institute (IVRI), Izatnagar, Bareilly, India.

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