Immunomodulatory Properties of *Tinospora cordifolia* in Carbon Tetrachloride Intoxicated Swiss Albino Mice

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Abstract

Effect of *Tinospora cordifolia* extracts on immunostimulatory functions in carbon tetrachloride (CCI₄) intoxicated Swiss albino mice is reported here. Administration of CCI₄ (0.05ml/kg bw for 7 days) caused immunosuppression as evident from splenic macrophage functions viz., phagocytosis, cell adhesion and myeloperoxidase release. Treatment with *T. cordifolia* extract (10 mg/kg bw for 15 days) in CCI₄ intoxicated mice ameliorated the immunosuppressive effect of CCI₄ since we found a significant increase in the cellular functions of murine splenic macrophages following *T. cordifolia* treatment. Our findings suggest that *T. cordifolia* may have a therapeutic role against immune toxicity.

Key words: *Tinospora cordifolia*, CCI₄, macrophage, immunomodulation

Introduction

*Tinospora cordifolia* (Guduchi) is a large deciduous climbing shrub belonging to the family Menispermaceae and is widely distributed throughout India. Previous studies have shown the pharmacological effects of *T. cordifolia*. Antineoplastic activity of this plant has also been reported (Jagetia and Nayak, 1998). Hepatoprotective and immunomodulatory effects of *T. cordifolia* have been reported in rats (Bishay et al, 2002). Oral administration of the extract of *T. cordifolia* roots for 6 weeks resulted in a significant reduction in blood and urine glucose and in lipids in serum and tissues in alloxan diabetic rats. The extract also prevented a decrease in body weight (Stanley et al, 2003). *T. cordifolia* is widely used in Indian ayurvedic medicine for treating diabetes mellitus (Stanley et al, 2001; Prince and Menon, 1999; Matthew and Kuttan, 1997).

There are very few reports on the immunomodulatory role of *T. cordifolia* along with its hepatoprotective activity, especially in mice. Since carbon tetrachloride (CCI₄) has been reported to cause liver and immune system damage, this model of inducing toxicity (Recknagel and Glende, 1973) has been used in the present study. The present study reports that treatment with *T. cordifolia* extract (10 mg/kg bw for 15 days) in CCI₄ intoxicated rats ameliorates the immunosuppressive effect of CCI₄ since we found a significant increase in the cellular functions of murine splenic macrophages following *T. cordifolia* treatment.

Materials and Methods

**Plant material**

*Aqueous extract of bark of Tinospora cordifolia* was used to study its immunomodulatory role.

**Animals and animal care**

Adult male Swiss albino mice with an average body weight of 20 g were used in the study. Animal care and protocols were in accordance and
approval by the institutional animal ethics committee. These animals were kept in an environment with controlled temperature (25°C), humidity (45-50%), and photoperiod (12:12-h light-dark cycle). All the animals were fed standard diet ad libitum and had free access to water.

**Treatment of animals and sample collection**

Animals were injected (i.p.) with CCl₄ with the dose of 0.05 ml/kg bw for 7 days (Recknagel and Glende, 1973). To study the effect of *T. cordifolia*, an aqueous extract of its bark was force-fed with a feeding needle (10 mg/kg bw for 15 days). On the 16th day, the mice were euthanized and spleen liver tissue dissected out for analysis.

**Separation of splenic macrophages**

Spleens were excised from killed mice and immediately placed in Alsever's solution and then macerated using frosted glass slides. Cells were repeatedly aspirated until a single cell suspension was obtained. The supernatant was then layered over Histopaque 1077 (Sigma, USA) and subjected to density-gradient centrifugation (Sikorski, et al, 1991). Thereafter, the band of leukocyte enriched fraction at the interface was collected and washed with DPBS, then the cell pellet was resuspended in RPMI-1640 containing 20 mM HEPES (pH 7.2). The non-adherent cells were removed and adherent cells were collected by repeated aspiration with a pasteur pipette. Cells were then washed and finally resuspended in culture media (RPMI + FBS) at a density of 10⁶/ml. More than 95% cells were found viable as determined by Trypan Blue dye exclusion technique. The cells were stained in Giemsa and observed under an oil-immersion microscope. Specificity of the cells was maintained by non-specific esterase staining.

**Phagocytosis assay**

100 μl spleen cells (10⁶ cells/ml) both from control and exposed groups were allowed to adhere separately on glass slides for one hour. Non-adherent cells were washed out with DPBS-1-X. 10% SRBC was added to the glass slides with the adhered macrophages. Slides were then incubated for three hours at 37°C. The cells were washed in DPBS-1-X and allowed to dry. They were then fixed in 50% methanol and stained by Giemsa. Cells were then observed and counted under oil immersion microscope. Phagocytosis Index (P.I.) was calculated using the formula: P.I. = % macrophages containing SRBC × average number of SRBC per macrophage × 10⁷ (Czuprynski . et al, 1984).

**In vitro cell adhesion assay**

100 μl cells (from 10⁶ cells/ml) were seeded separately for treated and control group in 96 well microtirte plate and allowed to adhere differentially for 0, 30, 45 and 60 min. With time, wells were washed with HBSS then 100 μl of 0.5% crystal violet, in 12% neutral formaldehyde and 10% ethanol was added to each well and incubated for 4 hr to fix and stain the cells, wells were then washed and air dried for 30 min. Crystal violet was extracted from the adhered macrophage in the wells by lysing with 0.1% SDS in HBSS. Absorbance was measured spectrophotometrically at 570 nm. Cell adhesion was expressed as the increased absorbance at 570nm (Lin. et al, 1995).

**Myeloperoxidase release assay**

200 μl cell suspension was taken and stimulated with LPS (100 ng/ml). Cells were incubated for 1 hour at 37°C. The cells were centrifuged and supernatant was collected in separate microcentrifuge tubes. The cells were lysed with 0.1% SDS and again centrifuged as before. The supernatants were once again collected in separate tubes. The cell-free supernatant was taken and allowed to react with OPD substrate in dark for 30 min. The blank was prepared with citrate phosphate buffer and substrate, in absence of cell-free supernatant. The reaction was then stopped with addition of 2(N) sulfuric acid and readings were taken at 492 nm in a spectrophotometer (Bos. et al, 1990).

**Statistical Analysis**

A one-tailed Student's t test was performed to compare the mean values of control and heavy metal-treated groups in the *in vivo* study.

- 36 -
Results

Effect of *T. cordifolia* administration on phagocytosis in CCl₄ intoxicated rats

![Fig.1.1](image)

The results in figure 1.1 represent the phagocytic index (mean ± SD). Phagocytic index of the control group was found to be 12255.25 ± 65.75 while that of CCl₄ intoxicated group was found to be 4545.5 ± 155.33 (P < 0.01). Administration of *T. cordifolia* in CCl₄ intoxicated mice led to increase in phagocytosis to 11893.0 ± 123.3 (P < 0.01). *T. cordifolia* treatment in control animals had no significant effect [Fig 1.1].

Effect of *T. cordifolia* administration on cell adhesion property in CCl₄ intoxicated rats

![Fig. 1.2](image)

Cell adherence was expressed as the increased absorbance at 570 nm. After 30 min incubation, absorbance in CCl₄-treated group was found to be 0.082 ± 0.0128 from 0.15 ± 0.054 of control group. After 60 min incubation, absorbance in CCl₄-treated group was found to be 0.132 ± 0.038 (P < 0.05) from 0.24 ± 0.055 of control group (P < 0.05). Administration of *T. cordifolia* in CCl₄ intoxicated mice led to increase in cell absorbance to 11893.0 ± 123.3 (P < 0.01). *T. cordifolia* treatment in control animals had no significant effect [Fig 1.2].

Effect of *T. cordifolia* administration on myeloperoxidase (MPO) release in CCl₄ intoxicated rats

![Fig.1.3](image)
CCl₄ intoxication reduces MPO release to 42.5 ± 1.75 % from 55.5 ± 2.25 % as in control group (P < 0.05). Administration of T. cordifolia in CCl₄ intoxicated mice increases the MPO release to 68.5 ± 3.45 % (P < 0.01). T. cordifolia treatment in control animals also increased MPO release to 69.45 ± 4.35 % (P < 0.01) [Fig 1.3].

Discussion

The present study was undertaken to determine the effect of Tinospora cordifolia extracts on immunostimulatory functions in carbon tetrachloride (CCl₄) intoxicated Swiss albino mice. We report that CCl₄ exert adverse effect on immune functions as evident from study of immune cell functions. However, in vivo administration of T. cordifolia extract caused immunoprotective activity in CCl₄-induced pathological manifestation of immunotoxicity.

Tissue macrophages arise by maturation of monocytes that have emigrated from blood and by proliferation matured into resident macrophages that retain their ability to recognize foreign materials. Differentiation of a monocyte into a tissue macrophage involved a number of changes. Macrophages are dispersed throughout the body. Some take up residence in particular tissues becoming fixed macrophages, whereas others remain motile and are called free or wandering macrophages. Macrophages are normally in a resting state, but in course of an immune response, a variety of stimuli activated macrophages.

Contact between particulate antigen and phagocytic cell was important for initiation of phagocytosis. The contact was brought about by transport of particles via blood or lymph to the sites of fixed phagocytic cells. CCl₄ may decrease the number of cells present in the spleen as well as altering the ability of those that remain to function normally. Depression of the oxygen dependent killing mechanism as determined by MPO release, portrays how CCl₄ negatively regulates the numerous functions of macrophage. Respiratory burst during phagocytosis resulted in activation of membrane-bound oxidase that catalyzed the reduction of oxygen to superoxide anion. It may be predicted that T. cordifolia, by increasing the level of MPO, might be serving a beneficial role as an antioxidant, and is capable of scavenging free radicals from a system or tissue, either by prolonging the initiation phase or by inhibiting the propagation phase of autooxidation. CCl₄ is known to cause hepatic injury and jaundice that often leads to an immunocompromised state that might facilitate opportunistic infections. T. cordifolia, having a significant immunostimulatory effect, has prevented pathogenic invasion.

References

