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EVALUATION OF IMMUNOMODULATORY EFFECT OF *TINOSPORA CORDIFOLIA* STEM AND ROOT IN MICE

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ABSTRACT

The present study aimed to evaluate immunomodulatory effects of stem and root aqueous extracts of *Tinospora cordifolia* (*TC*) in mice. *TC* creeped on neem plant was collected and extracts were prepared. A total of 18 Male Swiss Albino mice divided into three groups were used in the study. Group-I mice were treated with distilled water. Group-II mice treated with aqueous extract of *TC* stem (200 mg/kg), Group-III mice treated with aqueous extract of *TC* root (200 mg/kg). Physical and immunological parameters were measured at 2nd, 4th and 6th week. At the end of study mice were sacrificed under anesthesia. Thymus and Spleen was collected, weighed examined for histopathology. The result and findings of the study suggest the immune enhancement activity of stem and root aqueous extracts of *TC*.

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INTRODUCTION

Tinospora cordifolia (*TC*) is a climbing shrub belonging to the family Menispermaceae. The plant creeped on neem plant widely used in ayurvedic medicine because of its biological activities like anti-bacterial, anti- inflammatory, anti-diabetic, anti-oxidant, anti-allergic, anti-stress, anti-arthritis, hepatoprotective, imunomodulatory and various other medicinal properties (1-3). Considering these facts the present study designed to evaluate the immunomodulatory activity of stem and root aqueous extract of *TC* in mice models.

MATERIALS AND METHODS

Collection and Authentication of Plant Material: *Tinospora cordifolia* creeped on neem plant material (stem and root) was collected from Sanjeevapuram village, near Sri krishnadevaraya University Anantapuramu District, Andhra Pradesh state, India. The plant material was identified in the Botany department and voucher specimen (No:57412) was deposited in the SKU Herbarium, Department of Botany, Sri krishnadevaraya University, Ananthapuramu, India**.** Stem and root was dried under sunlight and made into fine powder by mechanical grinder. The powder was used for extraction.

Preparation of Aqueous Extract of plant material: Stem and root extracts of *TC* were prepared by cold maceration method (4). Stem and root of *TC* was dried and made into fine powder. 50gms of fine powder was weighed into sterile bottles and soaked in 500ml double distilled water and left for 48hrs at room temperature with intermittent shaking. The extracts were filtered using Whatman No.1 filter paper. The obtained extract was subjected to water bath evaporation at 60˚c temperature, semi solid *TC* extracts were obtained. The semisolid extracts were subjected to freeze drying. The extracts of stem & root obtained by this method were then weighed and percentage yield was found to be root 10%, stem 16%. The aqueous extract of stem & root were stored at 4˚c until further use.

Animals: Male Swiss Albino mice were used in this study. Male mice (25-30g) were procured from a registered Central Animal House (Sri Raghavendra Enterprises, Bangalore, Karnataka). The animal were housed in cage were kept under controlled conditions like temperature (25°C), sterilized rise husk bed and 12:12 h light and darkness cycles throughout the experimental period.

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The experimental animals have free access to standard pellet diet. Tap water was given on *ad libitum* (5). The University Ethics Committee (1889/GO/RE/S16/CPCSEA-30.05.2016) has approved the experimental protocol at Department of Microbiology, Sri Krishnadevaraya University, Anantapuramu, Andhra Pradesh, India.

Acute toxicity study of stem and root Aqueous extracts of *TC:* Male Swiss Albino mice (25-30g) were selected for acute toxicity study. The animals were fasted overnight and given the *TC* stem and root extracts at different doses (100, 200and 300mg/kg). According to study protocol, 2 mice per each dose were used. The graded doses were administered to their respective groups in morning time. After single dose administration, the animals were observed carefully for any signs of mild, moderate and severe (morbidity/mortality) behavioral changes for 24h (6). Administration of *TC* did not produce any mortality or any signs of behavioral changes or toxicity at the half of the maximum dose (200 mg/kg) was used for further studies.

Study groups

Evaluation of imunomodulatory study of stem and root aqueous extracts of *TC* in mice: Imunomodulatory effect of *TC* was investigated in albino mice using aqueous extract of stem and root. The details of treatment and groups were mentioned in table 1.

Table-1: Evaluation of immunomodulatory effect of stem and root aqueous extracts of *TC.*

Group	Drug Dose, Route of administration	Number of animals
Group-I	Control (10 ml Distilled water/kg)	6
Group-II	Aqueous extract of *TC* stem (200 mg/kg)	6
Group-III	Aqueous extract of *TC* root (200 mg/kg)	6

Procedure: Total 18 Male Swiss Albino mice were used in the study. Mice were divided into 3 groups each of 6 mice. Group-I mice were treated with distilled water, Group-II mice given Aqueous extract of *TC* stem (200 mg/kg), Group-III mice given Aqueous extract of *TC* root (200 mg/kg). All the mice receive extracts for 6 weeks. Physical and haematological parameters were measured every alternative week (2^{nd}, 4^{th} and 6^{th} week). Immunological parameters were measured on 6^{th} week. At the end of study period (6^{th} week) mice were sacrificed under anesthesia. Thymus and spleen were collected and weighed. To maintain the cell integrity and structure they were stored in bottles containing 10% formalin. Stored specimen were used for histopathological examination.

OBSERVATIONS

Physical parameters: Physical parameters were measured on alternative weeks (2^{nd}, 4^{th} and 6^{th}) during the study period.

Body weight (gm): Mice body weight was recorded by using digital electronic balance. The weight was expressed in mean gm.

Thymus weight (gm): The isolated thymus weight was measured by using electronic weighing machine. Thymus weight was expressed in gm.

Spleen weight (gm): The isolated spleen weight was measured by using electronic weighing machine. Spleen weight was expressed in gm.

Immunomodulatory parameters

Estimation of Humoral antibody response to Sheep Red Blood Cells (SRBC)

(Hemagglutination antibody titer test) (%): Blood sample was collected from healthy sheep and mixed with sterile Alsever's solution (1:1). The blood was then centrifuged at 1609.92×*g* for 5 min to enable red blood cells to settle at the bottom of the test tube. The supernatant was discarded, leaving the pellet of sheep red blood cells (SRBC). It was washed three times with pyrogen-free phosphate buffered saline (pH 7.2) and kept under refrigeration for use in the immunization challenge study. Mice were immunized by injecting 0.5ml of SRBCs intraperitonially (Figure 15) at the end of 6^{th} week. Blood samples were collected by retro orbital puncher. The collected blood was centrifuged at 2000 rpm for 10 min to get serum. Antibody titers were then determined by the hemagglutination technique (7). Two-fold serial dilutions of serum were made with normal saline in micro titer plates of 96-well capacity and SRBC (25 µL of 1% SRBC prepared in normal saline) added to each of these dilutions. The micro titre plates were then incubated at 37°C for 1 h and then examined for hemagglutination. The reciprocal of the highest dilution of the test serum giving proper agglutination was taken as the hemagglutination antibody titer and expressed as percentage (%).

Estimation of delayed type hypersensitivity (DTH) response (%): Mice paw model was used to determine the delayed type of hypersensitivity response. At the end of 6^{th} week, mice were primed by subcutaneous injection (0.1ml) with a suspension containing 1×10^8 SRBC at sub plantar region in the right hind footpad. The contra lateral paw also received an equal volume of 0.1% phosphate buffered saline (PBS). The extent of delayed-type hypersensitivity (DTH) response in the mice was determined by measuring the foot pad thickness after 0, 4, 8 and 24 h of challenge using a digital plethysmometer. The difference in the thickness of the right hind paw and the left hind paw was then used as a measure of DTH reaction and expressed as a mean percent increment in edema (8). It was calculated by using the following formula :

$$\% \text{ increment in edem} = \frac{|\text{Left foot pad challenged with antigen} - \text{Right food pad control}|}{\text{Left foot pad challenged with antigen}} \times 100$$

RESULTS AND DISCUSSION

Effect of *TC* stem and root on physical parameter: Body, thymus and spleen weight were taken as physical parameters. Body weight was measured on different time periods and compared. They were measured on 2^{nd}, 4^{th} and 6^{th} week. Control group showed increase in weight compared to standard and test groups as week's progress. Group-II and Group-III showed increase in the body weight compared to other groups. Co-administration of *TC* extract changes in body, thymus and spleen weight. Increase in the Group-II and Group-III lesser than Group-I. On 6^{th} week group-II showed significant ($p<0.04$) difference compared to other groups. Group-I not showed any significant difference. Within the groups also similar results were observed.

Group-II showed significant (p<0.001) increase in thymus and spleen weight compared to Group-I. Co-administration of plant extracts significantly changes in thymus and spleen. Group-III showed significant (p<0.001) difference compared to Group-II. Group-I compared to Group-II and III not showed any significant difference. Thymus and spleen was increased by co-administration of *TC* stem and root. In this study mice treated with *TC* stem and root showed increase in the body weight compared to control group. The 20-30% increase was observed between the weeks. Athar Husain *et al*., 2017 (9) observed that *TC* treatment increase the body weight. The present study results also showed similar effect. Veena Sharma *et al*., 2011 (10) study showed the effect of *TC* on body weight. The results showed increase in the body weight compared to control group. *TC* stem and root extracts contain various phytochemicals, which are responsible for the changes in body weight. In the present study similar effect was observed. *TC* stem and root increase the body weight.

Table 2. Effect of stem and root extracts of *TC* on body weight

Group	Body weight (g) (MEAN ± SD)		
	2nd week	4th week	6th week
Group-I	26.5 ± 1.33	27.4 ± 1.44	29.8 ± 1.44
Group-II	27.6 ± 1.11	28.2± 1.11	31.2± 1.64
Group-III	26. 2 ± 1.02	28.0± 1.83	30.5± 1.02

(*p<0.05 significant G-II)

Stem and root extracts of *TC* groups increased the thymus and spleen weight compared to control group. Thymus and spleen plays major role in the immune system. The major function of the thymus is in the maturation and selection of an antigen specific T- lymphocytes from bone marrow derived precursor cells (11) (Anderson *et al*.,1996). Spleen plays an important role in defense against blood-borne pathogens because it consists of Tcells, B cells, dendritic cells, red blood cells and macrophages. Changes in the thymus and spleen can affect the immune function of the body. Mice treated with *TC* changes in the thymus and spleen weights. These increases the T and B cell levels. This one of the reason stem and root extracts of *TC* increases the immune power of the body. Nageswari *et al*., 2018 (12) studied effect of *TC* on the spleen and thymus. In his study it was observed that rats treated with *TC* showed increase in the thymus and spleen weight. In the present study also similar results were observed. Bioactive compounds present the *TC* stem and root are responsible for this effect.

Table 3. Effect of *TC* stem and root extract on thymus weight

Group	Thymus weight (g) (MEAN ± SD)
I	0.18 ± 1.32
II	0. 26 ± 1.35
III	0.23 ± 1.56

(*p<0.05 significant Group-I, #p<0.05 significant Group-II)

Table 4. Effect of *TC* stem and root extract on spleen weight

Group	Spleen weight (g) (MEAN ± SD)
I	0.36 ± 1.20
II	0.67 ± 1.43
III	0.63 ± 1.10

(*p<0.05 significant Group-I, #p<0.05 significant Group-II)

In SRBC change test Group-II and Group-III showed significant (p<0.001) increase in the AB titer values compared to control group.

Mice treated with the stem and root extracts of *TC* significantly changes in SRBC challenge. The difference was statically significant. Stem and root extract of *TC* showed positive for steroids. The phytochemical may act as immunomodulator. Due its effect the AB titer value. Narkhede *et al*., 2014 (13) study showed the effect of *TC* on SRBC challenge on AB titer value. The results showed increase in the titer value compared to control group. Athar Husain *et al*., 2017 (9) studied the immunomodulatory effect of *TC*. They observed that co-administration of *TC* showed increase in the titer value. Sonavale *et al*., 2019 (14) demonstrated that administration of *TC* causes the increase in AB titer value compared to control group. The results were similar to our study showing increase in AB titer value in groups of mice treated with *TC* extracts. *TC* may contain chemicals that increase the immune cells and function. Increase in immune cells can lead to increase the titer value may be due to the effect of chemicals on immune system. Increase the immune cell function can increase the titer value. On the last day mice were subjected to SRBC challenge on AB titer. In this study mice treated with stem and root showed increase in titer value compared to control group. The effect of *TC* on SRBC challenge on AB titer value. The study results showed increase the titer value compared to control group. *TC* administration increased the titer value. In the present study also co-administration of *TC* stem and root increase the titer value compared to control group. This effect may due to stimulation on immune system.

Table 5. Effect of *TC* stem and root extract on SRBC challenge on AB titer values

Group	SRBC challenge on AB titer (MEAN ± SD)
I	80.41 ± 1.86
II	89.85 ± 1.40
III	87.90 ± 1.30

(*p<0.05 significant Group-I, #p<0.05 significant Group-II)

Group-I showed significant increase in paw volume compared to Group-II and Group-III. Co-administration of *T.C* stem and root extracts significant inhibited the increase in the paw volumes. *T.C* stem and root have immunomodulatory and anti-inflammatory effect. Those actions are useful to reduce the paw volumes. The differences between the groups is statically significant (p<0.001).

Table 6. Effect of *TC* stem and root extracts on mice paw volume

Group	Mice paws volumes (%) (MEAN ± SD)			
	0 h	4 h	8 h	24 h
I	07.12 ± 0.43	16.89 ± 1.54	25.85 ± 2.32	34.21 ± 0.21
II	06.31 ± 1.43	13.89 ± 0.88	21.32 ± 1.56	30.67 ±1.31
III	06.93 ± 0.23	14.29 ± 0.40	22.98 ± 1.31	31.86 ± 1.51

(*p<0.05 significant Group-I)

TC stem and root have effective source of immunostimulatory and anti-inflammatory agents for various infectious and anti-inflammatory diseases and there is growing interest in the development of therapeutic drugs of plant origin. Inflammation is the local immune response of tissue injury due to any agent. Edema is the initial sign of inflammation. In the initial phase serotonin and histamines are released, prostaglandins are released at the final phase of edema. Stem and root aqueous extract of *TC* suppress the edema formation. Administration of irritant in mice paw can stimulate immune response and increase the aggregation of immune cells. These immune cells release inflammatory mediators.

These mediators leads to increase in the paw volume. PGE2, IL-2, IL-6, TNF-alpha and IFN-gamma are the important inflammatory mediators in the immune system. Biswajyoti Patgiri *et al*., 2014 (15) studied the anti-inflammatory of *TC* and observed decrease in the paw volume. Siddalingappa *et al*., 2017 (16) also showed the reduction in the paw volume upon administration of *TC*. Steroids are commonly known as anti-inflammatory drugs, stimulates the immune cells and reactions. Accumulation and aggregation of immune cells decrease in presence of steroids. These can inhibits the synthesis of inflammatory mediators. Decrease the inflammatory mediators effect on paw volume. Several medicinal plant extracts contain various phytochemicals which are responsible for immunomodulatory activities to reduced the hyper inflammatory response of cytokines. *TC* showed positive test for steroid (17). Mice treated with *TC* stem and root reduces the paw volume. Similar effect was observed in our study indicating the immunomodulatory and anti-inflammatory effect of stem and root aqueous extract of *TC* in mice. This effect of aqueous extract of *TC* may be due to presence of anti-inflammatory substances such as steroids. These can reduces the inflammatory mediators and this effect may be useful in the mice paw model. Aqueous extract of *TC* stem and root may constitute anti-inflammators and produce the immunomodulatory and anti-inflammatory effect.

CONCLUSION

The present study was undertaken to evaluate the effect of *TC* on immunomodulatory activity. *TC* is a highly valued medicinal plant with diverse therapeutic uses in the traditional Indian systems of medicines such as Ayurveda, Unani and Siddha. It shows great potential as a safe and effective in immunomodulation. The review on Immunomodulatory herbs shows recent advancements happened in the research in these fields. This shows great potential of herbs to cure or prevent certain diseases.

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