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

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ABSTRACT: The present study aimed to evaluate haematological effects of stem and root aqueous extracts of *Tinospora cordifolia (TC)* in mice. *TC* encroaching on neem plant was collected and extracts were prepared. A total of 18 Swiss albino mice divided into three batches were used in the study. Batch I mice were treated with distilled water. Batch II Mice were treated with aqueous extract of *TC* stem (200mg/kg), Batch III Mice were treated with aqueous extract of *TC* root (200mg/kg). Haematological parameters were measured at 2nd, 4th and 6th week. The result and findings of the study suggest the haematological activity of stem and root aqueous extracts of *TC*.

Keywords : *Tinospora cordifolia*, haematology

INTRODUCTION

Plants the source of many potent drugs are used for various ailments for thousands of years all over the world. The use of natural products is as ancient as human civilization and there exists a magical, religious belief with regard to health in almost in every culture. At the initial stages, the western societies considered the use of natural products as superstitious by low income people with no pharmacological value. Now–a-days the scenario has changed and according to World Health Organization (WHO), 12 per cent of the world prescribed drugs is of natural origin. Among them 252 drugs were considered as basic drugs, of which 11 per cent are of plant origin. The natural products have the advantage of cost effectiveness without harmful side effects. For the discovery of new medicines, the natural products were considered as the single most successful strategy (Raskin et al., 2002).

Several plant products have valuable medicinal properties for the treatment of various chronic diseases like cancer, tuberculosis, aplastic anemia etc. *Tinospora cordifolia (TC)*, also known as Guduchi, is a plant in the Menispermaceae family that we used in our research. The versatile medicinal plant is a one-of-a-kind source of a wide range of chemically varied chemicals. The herb *TC* is from of a family of medicinal plants native to India's tropical and subtropical areas. The plant is widely utilised in Indian medicine; extracts from

various portions of the herb have been used to treat a broad range of ailments (Sengupta et al., 2011). Immunomodulatory components have been discovered in it. Giloy's many medical characteristics and therapeutic applications, as well as phytochemical research, demonstrate its value as a remarkable medicinal plant (Promila et al., 2017). *TC* is known to be beneficial in the treatment of the disorders like diabetics, peptic ulcers inflammatory disorders. It exhibits immunostimulant activity. Therefore *TC* is used for its imunomodulatory effect in our study. In ayurveda's *TC* is used as rasayana which has powerful immunostimulatory activity charaka described rasayana as anti-aging which increased the life span improved memory and freedom from diseases indicating immunostimulant effect.

MATERIALS AND METHODS

Collection of Plant material

Tinospora cordifolia was identified encroaching on neem plant systems (stem and root) in the Sanjeevapuram hamlet of the Anantapuramu District of Andhra Pradesh, India. The plant material was identified in the Botany department, and a voucher specimen (No: 57412) was put in the Herbarium of Sri Krishnadevaraya University, Department of Botany, Ananthapuramu, India.

EXTRACTION PROCEDURE

Grinding of selected materials from plants

The plant's stem and root samples were removed and carefully cleaned with running tap water to eliminate dirt particles and other pollutants before being rinsed with sterilised distilled water. Samples of stems and roots were dried for 72 hours at 37°C. To prevent the loss of active components, sunlight was avoided. The plant material was broken into bits after drying and processed into fine powder separately using a blender. The powder was kept in a safe place and utilised for various extractions and tests.

Preparation of aqueous *TC* stem and root extracts

Cold maceration was used to make the aqueous extract. Separate 100 g stem and root powders were steeped in 1000 ml 3 days at ambient temperature with periodic mixing with double distilled water. The extracts were filtered through a muslin cloth on the fourth day.

To eliminate surplus water, the extracted or filtered components were placed in a water bath. *TC* stem and root excerpts were positioned in a glass beaker and evaporated for 7-10 hours daily for 3-4 days in a water bath set to 60°C, until a semi-solid extracted liquid was formed. The semi-solid extracts were stored in a deep freezer at –20°C. When needed, doses of *TC* stem and root were made. The return on investment was computed and given as a percentage (per cent). The stem yield was determined to be 16.73 per cent and the root yield was 10.7 per cent. The dark brown aqueous extracts were kept at –4°C until they were used. Further research was carried out using the extracts that had been kept (Mahuya et al., 2011 and Prashant et al., 2011).

Animals used in Experiments

In this investigation, Swiss Albino mice were employed. Albino mice weighing 25-30 g were purchased from a registered Central Animal Shelter (Sri Raghavendra Enterprises, Bengaluru, Karnataka). Throughout the trial, mice were kept in the animal home under controlled conditions such as temperature (25°C), sterile rise husk bed, and 12:12 h light and darkness cycles. The usual pellet feed for the test animals is provided free of charge. There was unlimited access to clean drinking water. (Avnish et al., 2010).

The research was approved by the Institutional Animal Ethics Committee

The experimental procedure performed by one research scholar of the Sri Krishnadevaraya University, Anantapuramu, Microbiology Department, Andhra Pradesh, India. The University Ethics Committee accepted it (1889/GO/RE/S16/ CPCSEA-30.05.2016).

Acute toxicity assessment of aqueous extracts of *TC* stem and root

Male Swiss Albino mice (25–30 g) were utilised in the acute toxicity study. The mice were given different doses of *TC* stem and root extracts (100, 200 and 300 mg/kg) after fasting overnight. Two mice were utilised per treatment, as per the research protocol. In the morning, the graded dosages were given to their respective groups. All of the animals were observed for 24 hours after receiving a single dose to see whether any behavioural changes were mild, moderate, or severe (Lalitha et al., 2012). At half of the maximal dosage (200 mg/kg), administration of *TC* did not result in any deaths, behavioural abnormalities or toxicity. Table 1 shows the specifics of the therapy given to the various groups.

STUDY GROUPS

Table 1: Evaluation of haematological parameters of aqueous extracts of *TC* stem and root in mice was investigated

Group	Dose and route of administration of the drug	Animals in number
Batch-I (Control)	10 ml distilled water/kg/po as a control	6
Batch-II (Test-1)	Aqueous *TC* stem extract (200 mg/kg/po)	6
Batch-III (Test-2)	*TC* root aqueous extract (200 mg/kg/po)	6

PROCEDURE

The effects of *TC* on the immune system have been studied

Six mice per group (for a total of 18 animals) were utilised in this investigation. Three sets of experimental animals were created Distilled water was used to treat Batch-I. Aqueous extract of *TC* stem (200 mg/kg) was given to Batch II, while aqueous extract of *TC* root (200 mg/kg) was given to Batch III. For six weeks, all of the mice were given the appropriate medications. Haematological parameters were evaluated every other week (2^{nd}, 4^{th} and 6^{th} week).

Haematological parameters studied

Blood collection and serum separation

Blood was taken from each mice at the conclusion of the second, fourth and sixth weeks. Mice were administered 0.3 ml of ketamine to produce anaesthesia prior to blood collection since blood collection is easier and less uncomfortable for the animals under anaesthesia. To evaluate the haematological parameters, one to two ml of blood was drawn from each mice in EDTA coated tubes using the retro orbital capillary tube method.

The blood samples were centrifuged at 2000 RPM for 10 minutes to separate the serum. After centrifugation, the tubes were gently removed and the serum was carefully collected using a 2 ml syringe. The separated serum was kept at – 20°C in the refrigerator and utilised for analysis. (Siva kumar et al., 2011).

Hematological characteristics

The RBC, haemoglobin, WBC and platelet count were calculated using an Automated Hematology Analyzer (AHA) (Benjamin et al., 1982 and Godon et al., 2012). The Automated Hematology Analyzer uses an electrical impedance approach that depends on cells conductance changing as they pass through a tiny aperture. An electric impulse is produced by a change in conductance, which may be detected and recorded. This approach also enables for cell volume measurement and offers the sensitivity required for measuring and distinguishing cell types in blood.

Red blood cell Estimation (RBC)

At three different times, blood samples were taken from G-I, II and III mice (2^{nd}, 4^{th} and 6^{th} weeks). The RBCs in each blood sample were counted by the AHA. The RBC count was calculated as a mean $\times 10^6$ cells/mm^3.

Estimation of hemoglobin (Hb)

Blood samples were collected from G-I, II and III mice at three time periods (2^{nd}, 4^{th} and 6^{th} weeks). Each blood sample was tested for haemoglobin by the AHA. The Hb concentration was calculated as a mean of g dL^{-1}.

White blood cell Count Estimation (WBC)

At three different times, blood samples were taken from G-I, II and III mice (2^{nd}, 4^{th} and 6^{th} weeks). The WBCs in each blood sample were counted by the AHA. The WBC count was calculated as mean $\times 10^3$cells/mm^3 on average.

Neutrophil Count Estimation

At three different times, blood samples were taken from G-I, II and III mice (2^{nd}, 4^{th} and 6^{th} weeks). The WBC of each blood sample was calculated by the AHA. The count of neutrophils was represented as a mean 10^3cells/mm^3.

Eosinophil Count Estimation

At three different times, blood samples were taken from G-I, II and III mice (2^{nd}, 4^{th} and 6^{th} weeks). The WBC of each blood sample was calculated by the AHA. The count of eosinophils was represented as a mean 10^3cells/mm^3.

Basophil Count Estimation

At three different times, blood samples were taken from G-I, II and III mice (2^{nd}, 4^{th} and 6^{th} weeks). The WBC of each blood sample was calculated by the AHA. The count of Basophils was represented as a mean 10^3cells/mm^3.

Estimation of Monocytes

At three different times, blood samples were taken from G-I, II and III mice (2^{nd}, 4^{th} and 6^{th} weeks). The WBC of each blood sample was calculated by the AHA. The number of monocytes was represented as a mean 10^3 cells/mm^3.

Estimation of Lymphocytes

At three different times, blood samples were taken from G-I, II and III mice (2^{nd}, 4^{th} and 6^{th} weeks). AHA calculated the number of lymphocytes in each blood sample. The lymphocyte count was calculated as 10^3 cells/mm^3 on average.

Estimation of Platelet count

At three different times, blood samples were taken from G-I, II and III mice (2^{nd}, 4^{th} and 6^{th} weeks). AHA calculated the number of platelets in each blood sample. The platelet count was calculated as 10^3 cells/mm^3 on average.

RESULTS AND DISCUSSION

Effect of *TC* stem and root extracts on hematological parameters

Every other week, the number of red blood cells was calculated. When equated to Group-I, Group-II had a substantial ($p<0.03$) rise in RBC levels (Table 2). The amounts of RBCs alter dramatically when plant extracts are given together. *TC* stem and root treated groups presented significant growth in the RBC count in all the weeks compared to control group (Table 2). Group-II and III show significant difference compared to Group-I. RBC levels were improved by co-administration of *TC* stem and root extracts. Comparison of RBC count during 2^{nd}, 4^{th} and 6^{th} week exhibited significant difference ($p<0.02$).

Table 2: *TC* stem and root extract on red blood cells count effect in mice

Group	RBC (10^6 cells/mm^3) (MEAN ± SD)		
	2^{nd} week	4^{th} week	6^{th} week
Group-I	8.51 ± 0.46	8.84 ± 0.79	9.10 ± 0.46
Group-II	9.48 ± 0.13*	9.92 ± 0.46*	10.16 ± 0.24*
Group-III	9.15 ± 1.46*	9.72 ± 1.35*	10.00 ± 0.93*

(*$p<0.05$ significant Group-I)

In comparison to the control group, *TC* treatment increased Hb levels (Table 3). Hb levels were significantly altered when plant extracts were given together. There are substantial differences between Groups I, II, and III ($p<0.001$). A modest increase in Hb levels was detected after the 2^{nd}, 4^{th} and 6^{th} weeks. It is statistically significant to compare Hb values within the group. When related to Group I, Groups II and III revealed substantial differences.

Table 3: *TC* stem and root extract on haemoglobin effect in mice

Group	Haemoglobin (g dL^{-1}) (MEAN ± SD)		
	2^{nd} week	4^{th} week	6^{th} week
Group-I	12.26 ± 0.35	12.45 ± 0.35	12.85 ± 0.13
Group-II	14.10 ± 0.83*	14.35 ± 1.35*	14.75 ± 1.89*
Group-III	13.26 ± 1.46*	13.45 ± 1.89*	13.90 ± 1.23*

(*$p<0.05$ significant Group-I)

A assessment of WBC count inside the groups at 2^{nd}, 4^{th} and 6^{th} weeks displayed gradual increase which is statistically significant ($p<0.04$) with in the groups. Group-II and III showed significant difference when compared with Group-I (Table 4). The results of the control group were significant. When equated to the control group, *TC* administration increased WBC levels. Co-

administration of plant extracts resulted in a significant change in WBC counts. There are significant differences between Group-I and the test groups ($p<0.001$).

Table 4: *TC* stem and root extract on white blood cells effect in mice

Group	White Blood Cells ($\times 10^3$ cells/mm^3) (MEAN ± SD)		
	2nd week	4th week	6th week
Group-I	7.60 ± 1.68	7.95 ± 0.35	8.10 ± 0.13
Group-II	8.15 ± 1.46*	8.66 ± 0.13*	8.75 ± 1.35*
Group-III	8.01 ± 1.13*	8.10 ± 1.95*	8.15 ± 1.10*

(*$p<0.05$ significant Group-I)

A relationship of Neutrophils count inside group-I, II and III during 2nd, 4th and 6th weeks displayed gradual increase which is also statistically significant ($p<0.04$). Group-II and III displayed significant change with Group-I (Table 5). Both the control and test groups had statistically significant outcomes. In comparison to the control group, *TC* treatment boosted neutrophil counts. The addition of plant extracts to the diet caused considerable alterations in neutrophils. There are significant changes between Group-I and the experiment groups ($p<0.001$).

Table 5: *TC* stem and root extract on Neutrophils effect in mice

Group	Neutrophils ($\times 10^3$ cells/mm^3) (MEAN ± SD)		
	2nd week	4th week	6th week
Group-I	2.01 ± 0.92	2.19 ± 1.89	2. 23 ± 1.34
Group-II	2.25 ± 2.78*	2.33 ± 5.45*	2.49 ± 1.04*
Group-III	2.19 ± 2.78*	2.28 ± 9.45*	2.39 ± 1.14*

(*$p<0.05$ significant Group-I)

Counting Eosinophils in the sets throughout the 2nd, 4th and 6th weeks revealed a progressive rise. When equated to Group I, Groups II and III revealed substantial differences (Table 6). In comparison to the control group, *TC* treatment raised eosinophil levels. Co-administration of plant extracts caused in a considerable growth in the number of eosinophils.

Table 6: *TC* stem and root extract on Eosinophils effect in mice

Group	Eosinophils ($\times 10^3$ cells/mm^3) (MEAN ± SD)		
	2nd week	4th week	6th week
Group-I	0.35 ± 1.68	0.38 ± 0.35	0.39 ± 0.46
Group-II	0.43 ± 0.46*	0.45 ± 0.14*	0.46 ± 0.35*
Group-III	0.40 ± 0.14*	0.43 ± 0.93*	0.45 ± 0.12*

(*$p<0.05$ significant Group-I)

The number of basophils in each group increased gradually throughout the 2nd, 4th and 6th weeks. Groups II and III differed significantly from Group I. In comparison to the control group, *TC* treatment raised basophil levels (Table 7). Plant extracts administered together caused considerable alterations in basophils.

Table 7: *TC* stem and root extract on Basophils effect in mice

Group	Basophils (×10³ cells/mm³) (MEAN ± SD)		
	2nd week	4th week	6th week
Group-I	0.75 ± 0.93	0.78 ± 0.89	0.79 ± 0.35
Group-II	0.91 ± 0.46*	0.92 ± 0.35*	0.95 ± 0.55*
Group-III	0.81 ± 0.79*	0.83 ± 0.15*	0.86 ± 0.33*

(*p<0.05 significant Group-I)

During the 2nd, 4th and 6th weeks, a comparison of Monocytes in groups II and III revealed a progressive rise. Groups II and III differed significantly from Group I. (Table 8). Co-administration of plant extracts resulted in a considerable shift in the number of monocytes. There are significant changes between Group-I and the experiment groups (p<0.01).

Table 8: *TC* stem and root extract effect on Monocytes in mice

Group	Monocytes (×10³ cells/mm³) (MEAN ± SD)		
	2nd week	4th week	6th week
Group-I	1.35 ± 1.46	1.84 ± 0.13	1.81 ± 1.24
Group-II	1.53 ± 2.45*	1.64 ± 1.55*	1.73 ± 0.13*
Group-III	1.46 ± 2.33*	1.51 ± 1.44*	1.61 ± 1.57*

(*p<0.05 significant Group-I)

During the 2nd, 4th and 6th weeks, lymphocytes in groups I, II and III showed a steady rise that was statistically significant (p0.002). There was a substantial difference between *TC* stem and root extracts and the control. There were substantial disparities between Group-I and the test groups. When compared to the control group, *TC* treatment raised lymphocyte levels (Table 9). Co-administration of plant extracts resulted in a considerable shift in lymphocyte count. There is a significant change between Group-I and the experiment groups (p<0.01).

Table 9: *TC* stem and root extract effect on lymphocytes in mice

Group	Lymphocytes (×10³ cells/mm³) (MEAN ± SD)		
	2nd week	4th week	6th week
Group-I	5.13 ± 0.20	5.89 ± 1.24	5.93 ± 1.04
Group-II	7.19 ± 2.90*	7.85 ± 0.35*	7.95 ± 2.57*
Group-III	6.85 ± 1.57*	7.10 ± 1.09*	7.15 ± 1.93*

(*p<0.05 significant Group-I)

Platelet amount increased gradually in groups I, II, and III at the 2nd, 4th and 6th weeks, which was statistically significant (p0.002). There was a substantial difference between the *TC* stem and root extracts and the control (Table 10). There were substantial disparities between Group-I and the test groups. When compared to the control group, *TC* treatment boosted platelet counts. When plant extracts are given together, the platelet count changes significantly. There are significant changes between Group-I and the experiment groups ($p<0.01$).

Table 10: *TC* stem and root extract effect on platelet count in mice

Group	Platelet count ($x10^3$ cells/mm^3) (MEAN ± SD)		
	2nd week	4th week	6th week
Group-I	729.13 ± 1.35	735.23 ± 1.05	749.01 ± 1.94
Group-II	755.23 ± 1.94*	774.13 ± 0.46*	785.18 ± 2.19*
Group-III	735.13 ± 2.88*	750.94 ± 1.03*	770.12 ± 1.24*

(*$p<0.05$ significant Group-I)

The haematological parameters in the body were considerably changed by *TC* administration. *TC* has been shown to improve haematological parameters in several investigations. Sharma and Pandey (2010) looked examined the effect of *TC* on a variety of haematological parameters and found that it had a substantial impact on RBC, WBC and haemoglobin levels. *TC* causes increase in RBC, hemoglobin and other hematological cells. Vaibhav et al. (2010) demonstrated that alcoholic extracts obtained from dried ripe fruit of *Tinospora cordifolia* enhanced the bone marrow cellularity as well as α-esterase activity and serum immunoglobulin levels in the rats which evidently shows that it has potential activity on hematopoiesis. Romsha and Kalpana singh (2019) studied the *TC* effect on nicotin induced mice which showed that mice treated with *TC* showed significant increase in hematological parameters.

Nitin Verma et al. (2010) used *TC* to treat mice and looked at how it affected their haematological parameters. RBC, haemoglobin and other haematological cells were shown to be higher in *TC* treated animals. *TC* treatment induced considerable haematological alterations, according to Veena Sharma et al. (2011). They also discovered that *TC* had a considerable impact on platelets, neutrophils and lymphocytes. These findings suggest that *TC* has an immunomodulatory effect on hematopoiesis as evident from previous reports. In HIV-positive individuals, *TC* treatment is probably the most routinely utilised. There was a statistically significant rise in haemoglobin levels in *TC* clinical trial trials in individuals with advanced HIV. Romsha et al. (2019) studied the *TC* effect on alcohol induced mice for 60 days which showed significant increase in immune cells. Athar Husain et al. (2017) experimented the influence of *TC* on RBC and WBC count of mice. They observed that *TC* treated mice showed increase in RBC and WBC count. Similar reports with an increase in RBC and WBC count are in agreement with our data where *TC* treated mice have slightly increase RBC and WBC count as compared to control. Further, data suggest a drastic increase in haemoglobin level of *TC* treated mice as compared to control. Our data also suggest that *TC* may have a immunomoulatory effect in mice.

TC stem and root preparations are used to cure haematological problems, according to Ayurveda and accessible reports. Sharma et al. (2010) used *TC* at a dose of 400 mg/kg for 30 days. In comparison to the control group, they saw a rise in haematological parameter

values after the research period. Sudhakaran et al. (2006) showed the immunostimulatory effect of root ethanol and petroleum ether extract of *Tinospora cordifolia* on antibody response and neutrophil activity in *Oreochromis mossambicus*. Both the extracts had shown stimulatory effect on specific and non-specific defense mechanisms. Tinashe et al. (2012) discovered that herbal remedies are linked to a lower prevalence of ADRs, implying that herbals may provide some protection to antiretroviral therapy patients. It's worth noting that 98 per cent of patients used at least one herbal cure in addition to their highly active antiretroviral therapy (HAART) medicine.

CONCLUSION

From the above results, it can be concluded that aqueous extract of stem and root extracts of *TC* has haematological effect. These findings suggest that *TC* has immunomodulatory effect on haematopoiesis. Hence the study proved that *TC* stem and root preparations are used to cure haematological problems according to ayurveda and accessible reports.

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