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Effect of *Tinospora cordifolia* Leaf and Stem Aqueous Extracts on Zidovudine (ZDV) Induced Hematological Changes in Rat Model

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Abstract

The present study aims to evaluate the protective role of aqueous extracts of stem and leaves of *Tinospora cordifolia* (AELTC and AESTC) on the hematologic toxicity effects of ZDV in Rats. A Soxhlet extractor was used to prepare separate aqueous extracts of stem and leaf. Twenty-four albino rats (weighing 200-230 g) were separated into four groups. Group I rats were administered distilled water (negative control). Group II rats were administered 500 mg/kg b.w of ZDV (positive control), Group III rats were administered 500 mg/kg b.w. of AELTC along with ZDV, and Group IV rats were administered 500 mg/kg b.w of AESTC along with ZDV. The hematological parameters of the rats were assayed during the 2nd, 4th, 6th, and 8th weeks of treatment. The animals exposed to Zidovudine showed a significant decrease in Red Blood Cells (RBC), Hemoglobin (Hb), White Blood Count (WBC), and platelet count compared to control (Group I). The results of *T. cordifolia* leaf and stem administered rats showed a significant elevation in RBC, Hb, WBC, and platelets count. Spleen histology showed immunological and hemorrhagic changes with an accumulation of RBCs and severe depletion of lymphocytes in white pulp were observed in ZDV-treated rats. The intake of AELTC and AESTC showed apparent changes in Spleen tissues. These results suggest that AELTC and AESTC stimulate the hematopoietic system, thereby increasing blood cell levels in ZDV-treated rats.

Keywords: Immunotoxicity, Spleen and Haematology, *Tinospora cordifolia*, Zidovudine

1. Introduction

The use of herbal medicinal products and supplements has increased tremendously over the past three decades, with not less than 80% of people worldwide relying on them for some part of primary healthcare[1]. Currently, the use of traditional medicines worldwide is being re-valued through extensive research activity on various plant products and their therapeutic exploration. Recent study results showed that 80% of people living in developed countries still believe in traditional medicines for their health care management. They use plant products daily to keep the body healthy and fit[2]. Mono and polyherbal formulations are currently in demand, and their

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applications are increasing day by day due to their ease of availability and affordability. The detailed medicinal uses of 500 plants were explained in ancient literature. More than 800 medicinal plant products are being used in the management of various mild, moderate, and severe health disorders in humans[3].

T. cordifolia, commonly named Guduchi in Sanskrit, is a deciduous climbing shrub that belongs to the Menispermaceae family and is used to treat various diseases in traditional Indian literature. *T. cordifolia has* received attention in recent decades because of its utilization in folklore medicine for treating several disorders like cancer, jaundice, diabetes, urinary problems, stomachaches, prolonged diarrhea and dysentery. *T. cordifolia, a* deciduous climbing shrub indigenous to India has been used historically to combat acute and chronic inflammation as well as to promote a balanced immune response. *T. cordifolia* modulates vital signalling pathways related to cell proliferation, inflammation, and immunomodulation[4].

T. cordifolia is extensively used in Ayurveda due to its potential to improve the immune system and body resistance against infections. *T. cordifolia* is known as the queen of all herbs and was used in various studies to screen for its Immuno-modulatory, hematopoietic, anti-cancer anti-periodic, anti-spasmodic, anti-inflammatory, anti-arthritic, anti-allergic and anti-diabetic and anti-microbial activity[5]. The root and stem extracts of *T. cordifolia* showed anti-microbial activity against pathogenic microorganisms such as *Escherichia coli, Enterobacter faecalis, Klebsiella pneumonia, Staphylococcus aureus, Bacillus subtilis,* Streptococci. *T. cordifolia* has vast bio-diversity and traditional medicinal importance; it provides a new sight of challenging research for scientists to isolate pharmacologically active and therapeutic components from the plant to treat several dreadful diseases.

T. cordifolia exhibited potent immunomodulatory activity through stimulating pinocytosis and splenocyte proliferation. It also enhanced the subsets of various immune cells, mainly NK cells, B cells, CD4, and CD8 T cells, in cyclophosphamide-induced immunosuppressed mice. This immunomodulatory potential may be attributed to the presence of a group of metabolites such as phenols, flavonoids, tannins, alkaloids, and glycosides exhibiting multiple mechanisms[6].

T. cordifolia aqueous leaf and stem extract can be used for the treatment of opportunistic infections in HIV/AIDS patients[7]. Molecular docking studies revealed that *T. cordifolia* can be made as a drug for SARS Cov2 due to its anti-viral efficacy of phytochemicals like berberine, β-sitosterol, octacosanol, tetrahydropalmatine, and choline[8]. *T. cordifolia* extracts have been shown to result in up-regulation of IL-6 cytokine, resulting in acute reactions to injury, inflammation, activation of cytotoxic T cells, and B cell differentiation[9]. Administration of One gram of *T. cordifolia* leaf extract orally to the COVID-19-positive patients in the treatment group twice a day for seven days reduced inflammation markers and suggested less severity of SARS-CoV-2 infection in the treatment group[10]. *T. cordifolia* plant powder could be a potential alternative for non-steroidal anti-inflammatory drugs as the plant induces the secretion of interleukin-10 and interleukin-37 which subsidizes the interleukin-6[11].

2. Materials and Methods

2.1 Collection of Leaves and Stem of *T. cordifolia*

T. cordifolia leaves and stems were collected from local areas of Kothapalli village, Pamidi Mandal, Anantapuramu, Andhra Pradesh. The plant material was identified and approved by a botanist at the Department of Botany, Sri Krishnadevaraya University. Leaves and stem of *T. cordifolia* were shade-dried for five days. The dried contents were ground into fine powder separately. The powder was stored in a dark place to avoid losing active phytochemicals and used for different types of extractions and experiments.

2.2 Preparation of AELTC and AESTC

Aqueous extract was prepared using the cold maceration method[12,13]. The dark brown coloured aqueous extracts were stored at -4°C until use. The stored extracts were used for further studies.

2.3 Experimental Animals

The Wistar strain of Albino rats was used in this study. Male rats (150-200 g) were procured from a registered Central Animal House (Sri Raghavendra Enterprises, Bangalore, Karnataka). In the animal house, three rats per cage were kept under controlled conditions, such as temperature (25°C), sterilized rise husk bed, and 12:12 h light and darkness cycles throughout the experimental

period. The experimental animals have free access to a standard pellet diet. Tap water was given on *ad libitum*.

2.4 IAEC Approval of the Study

The study protocol was submitted to the Institutional Animal Ethics Committee of the Department of Pharmacology, RIPER, Anantapuramu, Andhra Pradesh. After a detailed discussion, the study was approved with the accession number 878/ac/05/CPCSEA/012/2013.

2.5 Acute Toxicity Study of AELTC and AESTC

Wistar Albino male rats (150–200 g) were selected for acute toxicity study. The animals were fasted overnight and given the AELTC and AESTC at different doses (200, 400, 800, and 1000 mg/kg). The graded doses were administered to their respective groups in the morning time. After single-dose administration, the animals were observed for any signs of mild, moderate, and severe (morbidity/mortality) behavioural changes for 24 hours[14]. Since no signs of toxicity were observed, half of the maximum dose (500 mg/kg) was used for further studies. We divided 24 adult male rats, weighing 200-230 g, into four groups, each with six rats placed in one cage. Group I was treated with 2 ml distilled water. Group II rats were administered 2 ml of 500 mg/kg b.w ZDV, Group III (test) was administered 2 ml of 500 mg/kg b.w AELTC along with ZDV (500 mg/kg b.w), and Group IV (test 2) administered 2 ml of 500 mg/kg b.w AESTC along with ZDV (500 mg/kg). All rats were administered respective drugs (excluding group I) for eight weeks. Hematological parameters of treated rats were assayed for every alternative week (2^{nd}, 4^{th}, $6^{th,}$ and 8^{th}) during the experiment.

2.6 Blood Collection and Serum Separation

Blood was collected from each rat at the end of the 2^{nd}, 4^{th}, 6^{th} and 8^{th} week. Before blood collection, rats were given 0.3 ml of ketamine to induce anaesthesia because blood collection under anaesthesia is accessible and less painful to the animals. One to 2 ml of blood was collected into the EDTA-coated tubes from each rat by retro-orbital capillary tube method to estimate the hematological parameters.

An Automated Hematology Analyzer (AHA) was used to estimate the RBC, WBC, hemoglobin, hematocrit, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and platelet count[15,16]. At the end of the study period (8^{th} week), rats were sacrificed under anaesthesia (Halothane). The spleen was collected and weighed. The specimens were stored in 10% formalin and used for histopathological examination.

2.7 Statistical Analysis

The results are expressed as mean±SD. The data were analyzed by one-way ANOVA followed by Dunnett's Test at the level of significance expressed as $p<0.05$ and $p<0.01$.

3. Results

3.1 Effect of AELTC and AESTC on Hematological and Histological Changes in Albino Rats

3.1.1 Hematological Studies

The hematological parameters were assayed in drug-treated rats (500 mg/kg b.w of each ZDV, AELTC, and AESTC) for every alternative week of the 2^{nd}, 4^{th}, $6^{th,}$ and 8^{th} during the experiment. The results of RBC count, Hb, WBC, and Lymphocytes shown in Figure 1, 500mg/kg b.w of ZDV, AELTC, and AESTC administration Group II animals (positive control) leads to significant ($p<0.05$) reduction in the RBC count, Hb, WBC, and Lymphocytes cells while compared with Group I rats. The RBC count, Hb, WBC and lymphocyte levels were significantly reduced and reached 4.90×10^{6} mm^3, 5.12 g/dL, 9.45×10^{3} mm^3, and 380.12×10^{3} mm^3 in the 8^{th} week of the study.

The results of Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Hematocrit are shown in Figure 2. Mean Corpuscular Volume (µm^3), Mean Corpuscular Hemoglobin (pg), percentage of Hematocrit and Reticulocytes were observed in ZDV

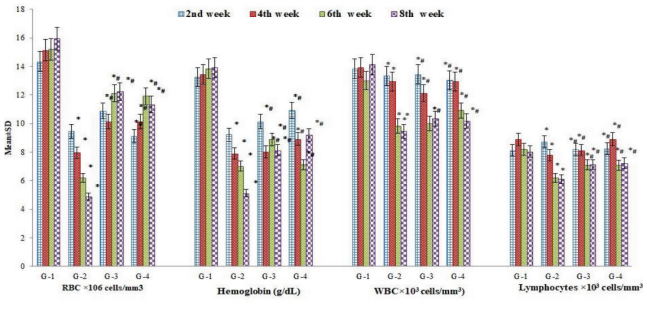


Figure 1. Effect of AELTC and AESTC on Red Blood Cell count (RBC), Hemoglobin (Hb), White Blood Cells (WBC), and Lymphocytes in ZDV-treated rats. (*$p<0.05$ significant Group I, #$p<0.05$ significant Group II).

treated rats as compared to a negative control group (Group I). Co-administration of 500 mg/kg b.w of AELTC and AESTC along with ZDV (Group III and IV) significantly ameliorated hematological cell content by nullifying the adverse effects caused by ZDV. These improvements in hematological conditions observed in AELTC and AESTC-administered rats suggest that *T. cordifolia* contains bioactive compounds that may stimulate hematopoiesis and serve as immune boosters.

The results of Reticulocytes, Neutrophils, Eosinophils, Basophils, Monocytes, and platelet count represented in Figure 3 and Table 1, ZDV treated rats Reticulocytes, Neutrophils, Eosinophils, Basophils, Monocytes and Platelet Count (PLT) were significantly increased when compared with the standard control group (Group I). The co-administration of 500 mg/kg b.w of AELTC and AESTC along with ZDV (Group III and IV), particularly Group III, significantly ameliorates hematological cell substance by reversing the toxic effects caused by ZDV. These improvements in Reticulocytes, Neutrophils, Eosinophils, Basophils, Monocytes, and platelet count observed in AELTC and AESTC-administered rats suggest that *T. cordifolia* contains a good number of immune-balancing bioactive compounds.

3.1.2 *Histopathology of Spleen treated with ZDV, AELTC and AESTC*

Histological examinations of sections of the spleen from Group III rats treated with the 2 ml of 500 mg/kg b.w of AELTC along with ZDV (500 mg/kg b.w) found ordinary

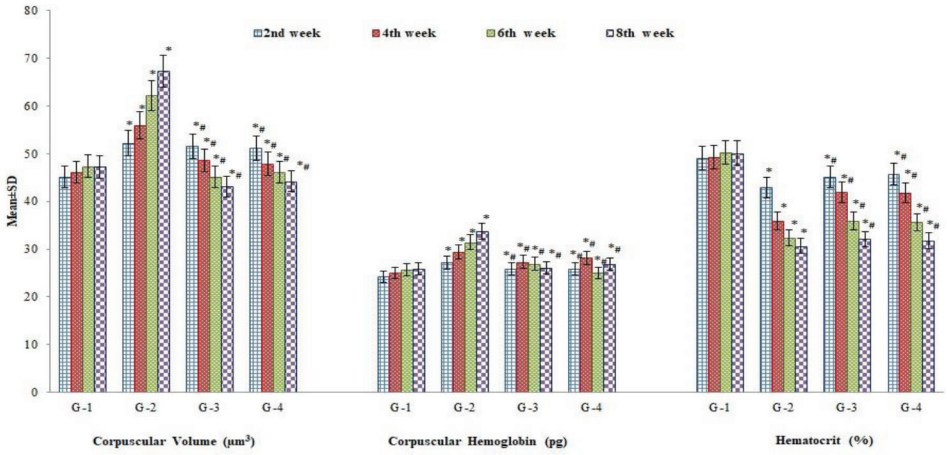


Figure 2. Effect of AELTC and AESTC on Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Hematocrit in ZDV treated rats. (*p<0.05 significant Group I, #p<0.05 significant Group II).

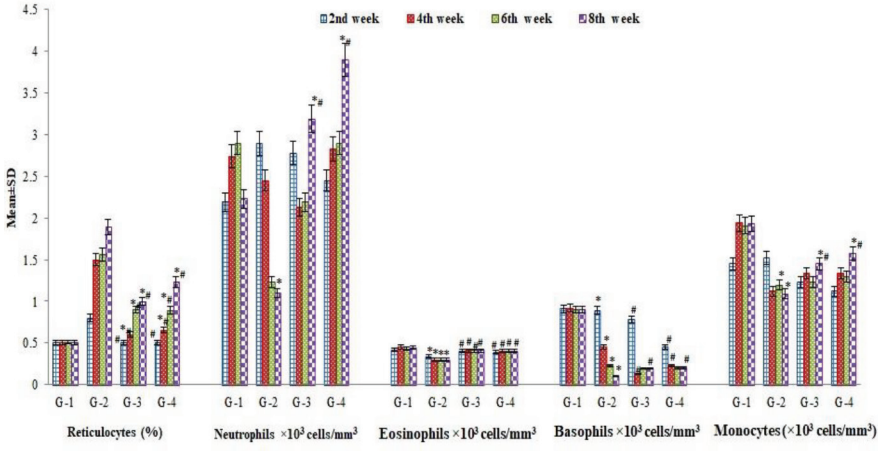


Figure 3. Effect of AELTC and AESTC on reticulocytes, neutrophils, eosinophils, basophils, and monocytes in ZDV treated rats. (*p<0.05 significant Group I, #p<0.05 significant Group II).

Table 1. Effect of AELTC and AESTC on Platelet Count (PLT) in ZDV-treated rats

Group	Platelet count (x10^3 cells/mm^3) (MEAN ± SD)			
	2nd week	4th week	6th week	8th week
I	740.12 ± 1.34	745.23 ± 1.04	749.01 ± 1.93	750.12 ± 2.12
II	564.04 ± 2.45*	449.12 ± 0.92*	400.19 ± 2.84*	380.12 ± 2.19*
III	600.23 ± 1.93*,#	550.13 ± 0.45*,#	600.18 ± 2.19*,#	670.12 ± 1.43*,#
IV	594.13 ± 2.89*,#	542.94 ± 1.02*,#	610.12 ± 1.23*,#	682.02 ± 2.81*,#

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

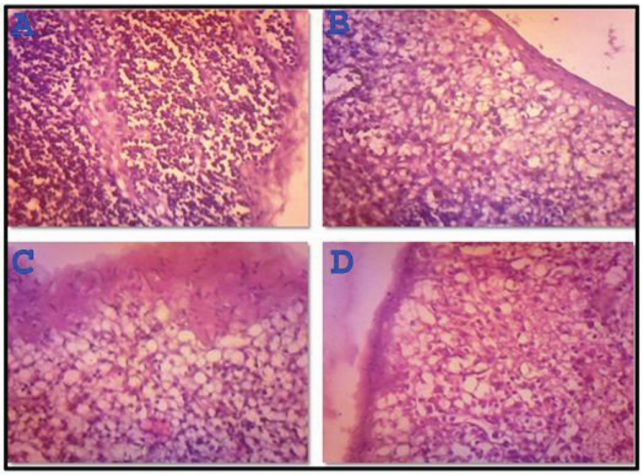


Figure 4. Effect of drug treatment on spleen histopathology. **(A)** Control group spleen, **(B)** Spleen treated with ZDV, **(C)** Spleen treated with AELTC, **(D)** Spleen treated with AESTC. All images are 400 x magnifications.

revealing lymphoid masses of white pulp (Figure 4(C)) while compared with Group II (2 ml of 500 mg/kg b.w of ZDV (Figure 4(B)) and Group IV (2 ml of 500 mg/kg b.w of AESTC along with ZDV (500 mg/kg b.w) (Figure 4(D)), when a highly vascular red pulp was compared to the spleens of control mice, it showed normal histology (Figure 4(A)).

4. Discussion

The significant adverse effect of Zidovudine (ZDV) is hematological toxicity, which results in anemia[17]. The critical difference in the RBC count and Hb levels was observed in ZDV injected rates compared to the control group. The RBC and Hb levels were further reduced and reached up to 4.90× 10^6 mm^3 and 5.12 g/dL in rats in the 8th week of the study (Figure 1).

Commensurate with the decrease in Hb levels, significantly lower RBC count in the ZVD injected rats compared to the control rats at the 6th and 8th week, indicating anaemic conditions. In the case of rats treated with AELTC and AESTC, the Hb levels were significantly higher compared to ZDV rats in the 6th and 8th week. In the clinical trial studies of ZDV in patients with advanced stages of HIV, a statistically significant reduction in haemoglobin levels occurred in 34% of subjects receiving ZDV (1,200 mg per day) following six weeks of therapy[18].

Anaemia in HIV patients is caused by a wide range of etiologic factors leading to decreased RBC production, increased RBC destruction, or ineffective RBC production. The decrease in RBC has been attributed to a reduction in erythropoiesis in the bone marrow and a faster rate of destruction of peripheral RBC in the spleen. Reduction in Hb can be related to the decreased size of RBC and impaired haem biosynthesis in bone marrow[19]. Our data suggest a drastic decrease in RBC, WBC, MCV, MCH, HB, and PLTs of ZDV-treated rats compared to control, indicating that ZDV intoxication caused hemolytic anaemia with various hematological changes (Figures 2 and 3). ZDV affects hematopoiesis, inhibiting heme and haemoglobin synthesis and inducing anaemia.

Compared to the control, AELTC and AESTC administration significantly prevented the ZDV-induced changes in RBC, WBC, MCV, MCH, HB, and PLTs. Hence, our findings confirm that *T. cordifolia* treatment benefits Hb levels and RBC count in ZDV rats. The research studies reported that administration of *T. cordifolia* leaf and stem methanolic extract to mice increased the total white blood cell count, bone marrow cellularity, and α-esterase positive cells in bone marrow, indicating increased maturation of stem cells[20].

In our study, total WBC decreased significantly from 13.83 × 10^3 mm^3 to 9.45 × 10^3 mm^3 in ZDV-treated rats compared to control. The rats supplemented with AELTC and AESTC significantly prevent ZDV-induced WBC changes. The significant decrease in the total indicates

that the ZDV possesses both dose and time-dependent leucopenic inhibitory effects on the progenitor cells in the bone marrow[21]. Thrombocytopenia is a condition that occurs when the platelet count in your blood is too low. The events of Thrombocytopenia are due to drug-induced adverse events or the infection itself. Thrombocytopenia may increase viremia, alter the immune system, and cause opportunistic infections and malignancies[22]. The platelet count decrease was also reported significantly from 564.04×10^3 mm^3 to 380.12×10^3 mm^3 in ZDV-treated rats compared to the control (Table 1). The rats supplemented with AELTC and AESTC significantly prevent ZDV-induced platelet count changes. The previous study also reported that extracts of *T. cordifolia* can enhance the platelet count within 15 days and normalize the platelet within 90 days of therapy[23].

The spleen is the lymphatic system's largest organ, which plays a role in immune functions such as recycling old red blood cells and serving as a repository for white blood cells and platelets. A histological examination of the spleen was conducted to understand the effect of AELTC and AESTC in rats treated with ZDV. Spleen showed immunological and hemorrhagic changes with accumulation of RBCs, and severe depletion of lymphocytes in white pulp was observed in ZDV-treated rates. The treatment with AELTC and AESTC protected the spleen from ZDV-induced changes by increasing the preservation of white pulp (Figure 4). The previous research reported that *T. cordifolia* was able to protect the spleen from HKBA-induced inflammation[24]. When the *T. cordifolia* extract and ZDV were administered, they decreased the toxic effect of ZDV in blood compared to ZDV-treated animals, thus indicating the protective role of the plant extract in ZDV toxicity.

5. Conclusion

We concluded from the above results that AELTC and AESTC have hematological and immunomodulatory effects. AELTC and AESTC restored hematopoiesis alterations caused by ZDV intoxication. Hence, the study proved that AELTC and AESTC preparations are used to cure hematological problems such as anaemia, hemophilia, leukaemia, lymphoma, and myeloma, according to Ayurveda and accessible reports.

6. Acknowledgement

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