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In vivo anti platelet activity of methanolic leaf extract of *Terminalia chebula* in experimental animals

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Abstract
The present research work investigates the effect of methanolic leaf extracts of *Terminalia chebula in vivo* by using wester albino rats. The acute oral toxicity of methanolic leaf extracts of the *Terminalia chebula* was studied by Lorke method. Lethal dose (LD 50) of the plant extract was entrenched and experimental rats were divided into 4 groups and then rats were administered with various concentration of leaf methanolic extracts (50 mg/kg, 100 mg/kg and 200 mg/kg) respectively. To analyze the hematological parameters, blood was withdrawn from the retro orbital puncture. The LD50 was calculated at 547.72 mg/kg. There was no significant change in hematological parameters ($p>0.05$) were observed when group I compared with group II which was administered with the dose of 50 mg/kg methanolic leaf extracts of the *Terminalia chebula.* While group III of which received100 mg/kg of plant methanolic leaf extract of *Terminalia chebula* showed significance ($p<0.05$) reduction in the packed cell volume (PCV), hemoglobin (Hb) and also reduction in the platelet count. At 100 mg/kg, the plant methanolic leaf extract showed significant anti platelet activity ($p<0.05$). The plant extracts at the dose of 200 mg/kg, showed significant increase in the concentration of hemoglobin, and indicates that can be used in the treatment of anemia. It can be concluded that the methanolic leaf extracts of *Terminalia chebula* exhibited hematinic, anti-platelet as well as anti-coagulation activity.

Keywords: Anti platelet activity, hematological, acute oral toxicity, *Terminalia chebula*

Introduction
Blood contributes homeostasis by transporting oxygen, carbon dioxide, nutrients and hormones to body cells and helps in regulates the body's pH, temperature and provides protection against various kinds of diseases through phagocytosis and the production of antibodies. Hemostasis, not to be confused with the similar term homeostasis means a sequence of responses that stops bleeding or arresting of hemorrhage. When blood vessels are ruptured or damaged, the hemostatic response must be quick, must be localized to regions of damage and carefully controlled in order to be effective. Three compensatory mechanisms play vital roles in reducing the blood loss from the ruptured blood vessels includes vascular spasm, platelet plug formation and coagulation or clotting of blood [1]. Using plants as herbal medicine by human beings for centuries. These herbal medicines and their derivatives possess different pharmacological and biological activities and are of a great role in modern health care systems [2]. Currently the demand and importance of herbal plants or medicines is growing worldwide day by day [3]. Demand of the new chemical entity for the development of health care system is highly supported by herbal plant resources [4]. Platelets respond to vascular injury by adhering to the endothelial matrix as a monolayer of cells and it is mediated by von Willebrand factor, a polymeric plasma glycoprotein that links platelets through surface glycoprotein Ib/IX receptors [5]. Throughout its lifetime in the circulation, the platelet is exposed to variety of external signals that regulate its state of activation [6] and most of these signals are mediated by binding to surface receptors and a series of amplifying mechanisms that converts external signal into cellular responses [7]. Anti-platelet drugs are used to treat and prevent various disorders of coagulation and thrombosis [8] and also stroke and ischemic attack [9]. A good antiplatelet drug should possess the characteristics like safety, efficacy and absence of adverse effects, a wide range of therapeutic window and low cost [10]. All anticoagulants and fibrinolytic drugs have adverse effects like excessive bleeding [11].

The present research study was performed to investigate the effect of leaves of methanolic extract of *Terminalia chebula* on blood coagulation and different blood parameters.

Materials and Methods

Collection of plant leaves: As this plant is widely distributed in southern region of India, the leaves were collected from Tirumala hills (Chittoor, Andhra Pradesh, India) and authentication of plant was done by Dr. K. Madhava Shetty, Taxonomist (Sri Venkateshwara University, Tirupati, Andhra Pradesh, India). A copy of the sample was preserved for future reference. All the solvents and chemicals were utilized in this work were procured from SD Fine Chemicals, Mumbai and Merck India, Mumbai.

Preparation of the extracts

The leaves were dried at room temperature (in air) and dried leaves were powdered, weighed (250 gm) for solvent extraction. The powdered plant material was macerated (for 24-72 hrs) and subsequently extracted with 1 liter of methanol. Resulted leaf extracts were concentrated using a rotary evaporator (in vacuo) and were stored in cold conditions for further investigations [12, 17].

Animal study

The experimental animals used in the present research were albino Wistar rats weighing between 120 -150 mg each. The experimental rats were procured from registered breeders (Venkateswara Enterprises, Hyderabad). A total number of 26 rats were selected (1-2 months old) for this research study. The animals were placed under standard conditions of room temperature (20- 25 ºC) and relative humidity (30-70%) with a 12:12 light-dark cycle. The animals were fed with standard pellet diet and water *ad libitum*. The norms for Good Laboratory Practice (GCP) were followed for care of laboratory animals. The animals were maintained in accordance with the CPCSEA guidelines. To distinguish each treatment group, the animals were marked with solution of picric acid.

Determination of acute oral toxicity study (LD50)

By using Lorke method, the acute oral toxicity of methanolic leaf extract of *Terminalia chebula* was performed [13]. A total number of 20 animals were used for conducting acute oral toxicity tests. This method consists of two phases, phase-I and Phase-II. In phase I, 12 experimental animals were required. These 12 experimental animals were grouped into three and four animals in each. Then the animals were administered with various doses of methanolic leaf extracts of (10, 100 and 1000 mg/kg) test substance through oral route of administration (*Terminalia chebula*). The animals are observed for 24 hours to identify their behavior as well as death. Phase-II involves the use of 8 animals, which are distributed into four groups of two animals in each. The animals are administered higher doses (1600, 2900 and 5000 mg/kg) of test substance and then observed for 24 hours for behavior as well as mortality. From the result or observation of phase I, the phase II tests were carried out. In the second phase animals were divided in to 4 groups and 2 in each. Group I received 500 mg/kg of plant extract through intraperitoneal route. Group II received 600 mg/kg while group III and IV received 700 mg/kg and 800 mg/kg respectively. The animals were monitored for 24 hours, and the number of deaths was noted.

Study design for *in vivo* anti platelet activity

A total number of 13 animals were selected for the *In vivo* anti platelet activity and animals were divided in to 4 groups. In group I, four animals were placed and in group II, III and IV three animals in each. Group I administered with normal distilled water through orally, whereas group II administered with 50 mg/kg of plant extract. Group III and IV received test doses of 100 mg/kg and 200 mg/kg plant methanolic leaf extract of *Terminalia chebula.*

Collection of samples: Animals were anesthetized with chloroform and blood samples were obtained from retro orbital puncture and blood was poured in to EDTA and Sod. Chloride tubes for hematological tests. After six hours of collection, the samples were analyzed by using standard methods [14, 15, 16].

Statistical Analysis: Data were expressed as mean ± SD. Statistical analysis was assessed using one-way analysis of variance. The significance level was considered (p<0.05).

Results and Discussion

In the phase I, no deaths were recorded in 10 mg/kg, 100 mg/kg group but in 1000 mg/kg group all the animals were died. In phase-II, death of animals was recorded at 600 mg/kg concentration of plant leaf methanolic extract of *Terminalia chebula* and no death at 500 mg/kg. Therefore, Minimum lethal dose 50 (MLD50) was found to be 547.72 mg/kg.

Table 1: The result of acute oral toxicity studies (LD50)

S. No	MLETCB dose	Number of deaths
Phase I		
Group I	10 mg/kg	0/4
Group II	100 mg/kg	0/4
Group III	1000 mg/kg	4/4
Phase II		
Group I	500 mg/kg	0/2
Group II	600 mg/kg	2/2
Group III	700 mg/kg	2/2
Group IV	800 mg/kg	2/2

Note: LD 50= Lethal dose 50, MLETBB= Methanolic leaf extract of *Terminalia chebula* LD50= √axb

Where a = highest dose that does not produce death, b= lowest dose that produced death.

= √500*600

= 547.72mg/kg

Table 2: Represents the Mean ± SD of the hematological result of 50 mg/kg, 100 mg/kg and 200 mg/kg of plant leaf methanolic extract of *Terminalia chebula* control (0.4 ml of distil water) and test in experimental albino wester rats

	Hematological parameters							
Groups	**PCV**	**ESR**	**RPV**	**PWBV**	**PFC**	**HB**	**WBC**	**PLTS**
Group I Normal control	38.00±1.55	2.01±0.75	1.17±0.27	1.39±0.08	0.87±0.32	14.10±2.98	3.89±0.68	333000±22380
Group 2 50mg/kg	37.01±2.98	2.04±1.19	1.19±0.20	1.33±0.02	0.77±0.30	15.80±3.96	4.30±0.61	295000±17650

Group 3 100mg/kg	36.00±2.21	2.01±1.51	1.08±0.04	1.29±0.08	0.87±0.03	12.48±0.82	3.60±0.82*	26300±18570*
Group 4 200mg/kg	35.90±1.56	2.02±0.59	1.19±0.75	1.39±0.08	0.81±0.22	17.89±1.45*	5.22±0.47	289000±10810*

Conclusion

The acute oral toxicity of the plant leaf methanolic extract of *Terminalia chebula* was determined by albino wester rats. In phase I, the plant leaf methanolic extract was given to the rats at different concentrations of 10 mg/kg, 100 mg/kg and 1000 mg/kg respectively. 10 mg/kg, 100 mg/kg of leaf methanolic extract failed to record death while 1000 mg/kg of leaf methanolic extract showed death of all the rats in the group. In phase-II, doses of plant extracts, as 500 mg/kg, 600 mg/kg, 700 mg/kg and 800 mg/kg of was administered to experimental rats. The MLD50 was calculated by the given formula which was indicated in (table 1).

In vivo thrombolytic activity was carried out by using albino wester rats. Group I served as normal control and was given 0.4ml of normal saline while group II received 50mg/kg of plant leaf methanolic extract of *Terminalia chebula*. When the two groups were compared, there is no significance in hematological parameters. This indicates that 50 mg/kg concentration of plant leaf methanolic extract of *Terminalia chebula* is well tolerated and hence no changes were recorded in hematological parameters. Whereas the concentration of 100 mg/kg of plant leaf methanolic extract of *Terminalia chebula* showed significance reduction in the packed cell volume (PCV), hemoglobin (Hb) and also reduction in the platelet count. At 100 mg/kg, the plant leaf methanolic extract showed significant anti platelet activity (p<0.05). The plant extracts at 200 mg/kg, exhibited that increase in the concentration of hemoglobin, and indicates that can be used in the treatment of anemia (Table 2). It can be concluded that the methanolic leaf extracts of *Terminalia chebula* showed hematinic as well as anti-platelet activity, this could probably justify the use natively as medicinal plant.

Conflict of interest

The authors declare that there is no conflict of interest.

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