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Preclinical Evaluation of Hydro-Alcoholic Extract of *Tinospora cordifolia* Leaves on Thrombocytopenic Wistar Rats

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Abstract

Objective: The main purpose of this study is to evaluate the ability of increasing the platelet count in rat model using hydro alcoholic extract of *Tinospora cordifolia* leaf based upon the pre existing pharmacological activity of that leaf such as antioxidant, antimicrobial properties.

Method: The collected leave was extracted by double maceration process using 50% ethanol. To evaluate the thrombocytogenic activity of the extract, cyclophosphamide induced and Chloramphenicol induced thrombocytopenia in rats were employed. The animals were divided into five groups and prednisolone was served as standard drug for both models. Haemocytometer was used to count the platelet. Bleeding time was also evaluated.

Result: In the cyclophosphamide induced thrombocytopenia in rat model prednisolone, *Tinospora cordifolia* low dose (TCLD) and *Tinospora cordifolia* high dose (TCHD) on 14th day has shown the number of platelet 1246583,903014 and 997078 respectively and in the Chloramphenicol induced thrombocytopenia in rat model perdnisolone, TCLD and TCHD on 24 hour has shown the number of platelet were 1124733,899720 and 903391 respectively while compared with toxicant control.

Conclusion: From the above findings it is cleared that *T.cordifolia* has shown significant increase in number of platelet count while compared to the toxicant control. Therefore it is concluded that *T.cordifolia* leave extract has significant thrombocytogenic activity.

Key Words: chloramphenicol, cyclophosphamide, haemocytometer, platelet count, prednisolone, *Tinospora cordifolia*.

INTRODUCTION

Platelets or thrombocytes are a component of blood which is responsible for blood clotting to stop bleeding at the injury site. Platelet does not contain any cell nucleus and inactivated platelet is biconvex in shape and 2-3µ in diameter. They are basically fragments of cytoplasm which are derived from the megakaryocytes of the bone marrow; cell membrane projections are present in activated platelets which covers their surface. [1]

Low platelet concentration in blood is called thrombocytopenia which occurs due to decreased production or increased destruction. This condition ranges from mild to severe depending on its underlying causes, such as certain medications, leukemic, bone marrow problems etc.

Sometimes, in case of pregnancy or some mild cases platelet count becomes low but usually it doesn't produce any symptoms. When it becomes more severe may cause uncontrollable bleeding or internal bleeding which requires immediate medical attention. The medical treatments which required in severe cases are blood or platelet transfusions, change in medications causes low platelet count, steroids, immune globulin, and corticosteroids to block platelet antibodies, drugs that suppress your immune system, spleen removal surgery which are expensive or pain full.[2]

Tinospora cordifolia also known as giloy is heart shaped leaf belonging to the family menispermacea[3] .This drug is known as queen of all medicinal plants and is prescribed as anti diabetic [4], anti oxidant [5], anti cancer [6, 7], anti microbial [8], anti toxic [9], anti HIV [10]. It also shows immunomodulatory effect [11]. Based on these beneficial properties of *Tinospora cordifolia*, the leaf extract is used to study the effect of platelet count on albino wistar rats.

MATERIALS AND METHODS

Animal: Albino Wistar rats weighing 150g both sex (male and female) were taken for the experiment and placed in polypropylene cages (32 X 24 X 16) cm. The animals were purchased from an authorised animal breeder and kept in CPCSE approved well maintained NSCBIP animal house (approval no: 1502/PO/a/11/CPCSEA), under standard hygienic conditions, at a temperature (22±2°C), 65% relative humidity, and maintained 12 hours light and dark cycle. They are provided with commercial food pallets and tap water ad libitum. All the experiments were held between 10 am to 6 pm.

Collection of sample: Green leaves were collected and washed gently with fresh water for removing the unwanted particles and good ones were shed dried. Then the dried leaves were made coarse by hand and subject to extraction.

Plant extract: The dried leaves were macerated twice by using 50% ethanol, which was divided into two parts in such a manner that the same volume was used both maceration. The whole process of double maceration took 72 hours to complete. First step took 48 hours and next step took 24 hours. The extract was collected and kept under vacuum desiccators for evaporation.

Preliminary phytochemical screening of the extract [12]: Ethanol extract of *Tinospora cordifolia* leaves were subjected to phytochemical investigation such as alkaloids, carbohydrates, tannins, saponin, glycosides, flavonoids, proteins etc.

Acute oral toxicity studies: The acute oral toxicity study was performed according to oppts (Office of prevention, pesticide and toxic substance) guidelines [13].

Statistical analysis: The experimental data were expressed as mean ± SEM for each treatment group. The significance of activity was assessed using a one-way analysis of variance followed by Dunnett's post-parametric test between the data of control and treated groups. *p<0.05 was considered statistically significant.

Dose preparation: 37.5 mg of extract was dissolved in 3.75 ml of distilled water to prepare 250mg/kg of stock solution. Similarly 75 mg of extract was dissolved in 7.5 ml of distilled water to prepare stock solution of 500mg/kg. Prednisolone, cyclophosphamide and chloramphenicol were dissolved in distilled water to prepare the solution 200mg/kg, 50mg/kg and 50mg/kg respectively.

Drugs and chemicals: Ethanol (LOBA CHEME PVT. LTD.), EDTA solution (lab made), Distilled water, Rees-Ecker fluid, prednisolone (Wysolone 5 mg by Wyeth Lederle Limited), cyclophosphamide (Endoxan 50 mg by Zydus oncoscience), Chloramphenicol.

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Experimental design

Model 1: cyclophosphamide induced thrombocytopenic rat: The rats were divided into five groups and each group contains six animals. To induced thrombocytopenia, the rats of groups II, III, IV, and V were treated with cyclophosphamide 50 mg/kg s.c. for three consecutive days.

Group I: These group animals received normal water p.o. throughout the experimental period. This group was considered as normal group.

Group II: This group was considered as toxicant control group and treated with cyclophosphamide 50mg/kg s.c. for first 3 days daily.

Group III: This Group received prednisolone 200mg/kg p.o. for 13 days daily along with cyclophosphamide 50mg/kg s.c. for first three days. This was considered as standard group.

Group IV: This was test group I and received extract solution *Tinospora cordifolia* low dose (TCLD) 250mg/kg, p.o. for 13 days daily along with cyclophosphamide 50mg/kg s.c. for first three days.

Group V: This was test group II and received extract solution *Tinospora cordifolia* high dose (TCHD) 500mg/kg, p.o. for 13 days daily along with cyclophosphamide 50 mg/kg s.c. for first three days.

Blood samples were collected on day 1, 4, 7, 10 and 14 from each group of animals and platelet count was done by haemocytometer. On 14th day bleeding time was determined.

Model 2: chloramphenicol induced thrombocytopenic rat: A total 6 days treatment period was conducted and animals were treated as follows:

Group I: Normal group: animals of this group received only water, p.o. throughout the experimental period.

Group II: Toxicant control group: animals of this grouped received Chloramphenicol 50 mg/kg p.o. on the 6th day of experiment.

Group III: Standard group 1: animals of this group received prednisolone 200 mg/kg, p.o. for consecutive 5 days followed by Chloramphenicol 50 mg/kg, p.o. 6th day

Group IV: Test group 1: animals of this group received TCLD 250 mg/kg, p.o. for consecutive 5 days followed by Chloramphenicol 50 mg/kg, p.o. on 6th day.

Group V: Test group 2: animals of this group received TCHD 500 mg/kg, p.o. for consecutive 5 days followed by Chloramphenicol 50 mg/kg, p.o. on 6th day.

On 6th day, after completion of all treatment, blood samples were collected at 0, 1, 4 and 24 hours intervals and platelet count was done by using haemocytometer. Bleeding time was measured.

RESULTS

Extract preparation: The leaves of *Tinospora cordifolia* were subjected to double maceration using 50% ethanol for consecutive 72 hours and 3.33% yield was obtained.

Preliminary phytochemical investigation: The extract obtained from the leaves of *Tinospora cordifolia* was subjected for preliminary phyto-chemical investigation and the following observations were found Table 1.

Table 1: A preliminary phytochemical investigation of *Tinospora cordifolia* leaves extract

Sl No.	Test names	Results
1	Test for alkaloids	positive
2	Test for glycoside	positive
3	Test for tannin	positive
4	Test for protein and amino acids	positive
5	Test for flavonoids	positive
6	Test for steroids and triterpinoids	positive

Acute oral toxicity studies: According to the OPPTs guidelines drug found to be safe at 2000mg/kg body weight.

Cyclophosphamide induced thrombocytopenia in rat: Cyclophosphamide is an immunosuppressant drug generally used to treat different types of cancer in fact this drug act by stopping the cell growth and majorly caused thrombocytopenia as a side effect [14]**.** "Cyclophosphamide induced thrombocytopenia in rat model" the animal were divided at received treatment as per their group belongs to. Prednisolone acted as a standard drug in this study. TCLD and TCHD as increased the platelet count from day 4 to day 14 in dose dependent manner. Prednisolone, TCLD and TCHD on day 14 have shown the number of platelet 1246583,903014 and 997078 respectively. The entire three groups have shown a significant level of increase in the number of mean platelets while compared with toxicant control.

Table 2: Mean platelet count with days in model 1

Treatment groups and dose (mg/kg)	Mean platelet count(cells per microliter) and Time interval in days				
	1st day	4th day	7th day	10th day	14th day
Normal control	933413 ± 23233	937004 ± 23395	932657 ± 19703	930211± 24525	934323 ± 18373
Toxicant control (cyclophosphamide, 50mg/kg)	931726 ± 27320	726503 ± 28044	438906 ± 16093	386525 ± 31114	397071 ± 26522
Standard Group (prednisolone, 200mg/kg)	940772 ± 22216	810513 ± 20488**	1231590 ± 21522***	1221446 ± 33709***	1246583 ± 8089***
Test group I (TCLD, 250mg/kg)	933616 ± 21046	711836 ± 26019	730682 ± 25922***	850770 ± 13118***	903014 ± 19071***
Test group II (TCHD, 500mg/kg)	926821 ± 21013	736046 ± 14091	798113 ± 5431***	886225 ± 16814***	997078 ± 18373***

All values are mean ± SEM, n=6, **P<0.01, ***P< 0.001 when Experimental groups compared with normal control

Table 3: Bleeding time of rats on day 14

	Mean bleeding time (sec) on day 14				
Groups	Normal control	Toxicant control	Standard group	TCLD	TCHD
Time(sec)	93±3.86	180±9.71	78±6.71***	103±4.46***	118±5.33***

All values are mean ± SEM, n=6, P values: *** < 0.001when Experimental groups compared with untreated (normal control) group

Table 4: Mean platelet count with hours in model 2

Treatment groups and dose (mg/kg)	Mean platelet count(cells per micro liter) and Time interval in hour			
	0 hour	1 hour	4 hour	24 hour
Normal control	942914 ± 29266	927784 ± 14013	939997 ± 16926	943303 ± 18341
Toxicant control (Chloramphenicol, 50mg/kg)	926523 ± 32611	762914 ± 19517	676633 ± 8526	655140 ± 19010
Standard Group (Prednisolone, 200mg/kg)	1240536 ±27014**	930771 ±31016***	1090519 ± 25032***	1124733 ± 16042***
Test group I (TCLD 250mg/kg)	1034013 ± 16213***	813902 ±19506**	846221 ±18321***	899720 ± 22063***
Test group II (TCHD, 500mg/kg)	1177525 ± 19582***	840613 ±20171***	865346 ±14516***	903391 ± 18809***

All values are mean ± SEM, n=6, **P<0.01, ***P< 0.001 when Experimental groups compared with normal control

Table 5: Bleeding time of rats on day 6

	Mean bleeding time (sec) on day 6				
Groups	Normal control	Toxicant control	Standard group	TCLD	TCHD
Time (sec)	90±4.71	175±10.37	80±6.91***	99±5.66***	113±6.1***

All values are mean ± SEM, n=6, ***P< 0.001 when Experimental groups compared with normal control

As the number of platelets is directly influence in the bleeding time and clotting time, evaluation of bleeding time in rats on day 14 has clearly given the idea of platelet generation. In toxicant control group cyclophosphamide has increased the bleeding time compared to untreated control. Prednisolone, TCLD and TCHD have shown significant acceleration on clotting while compared with toxicant control.

Chloramphenicol induced thrombocytopenia in rat: Chloramphenicol, an antibiotic is reported to cause thrombocytopenia by interfering with the protein

formation in thrombocyte[15]. In the present study "chloramphenicol induced thrombocytopenia in rat" has been introduced to evaluate the thrombocytogenic activity with more precision, as the number of more models eliminates the chance of errors compared to a single model. In this model the single dose of Chloramphenicol has previously damaged the thrombocyte in all the groups as the toxicant control has not received any protect drug on 24 hour it has shown the reduction of thrombocyte at 625140 only. On the other hand prednisolone, TCLD and TCHD have shown a remarkable increase in the number of mean platelets while compared with toxicant control. They were 1124733, 899720 and 903391 respectively.

Bleeding time of prednisolone, TCLD and TCHD have shown significant results on the 6th day while it compared with the toxicant control group.

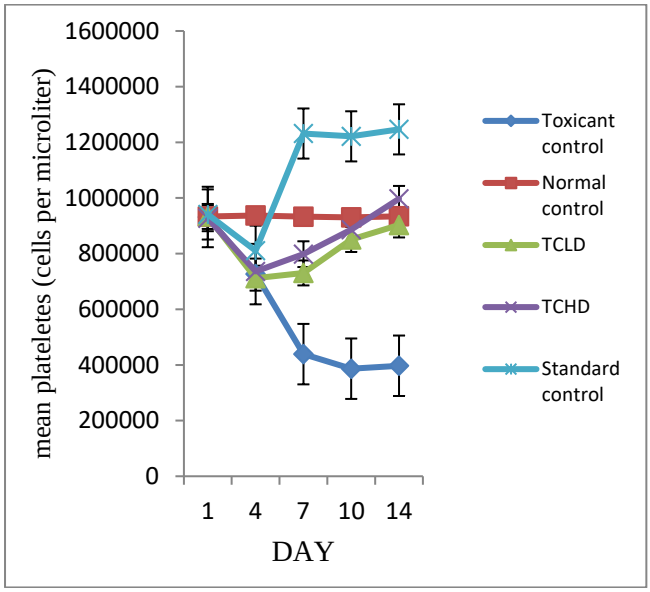

Figure 1: Graphical representation of mean platelet count with days (Model 1)

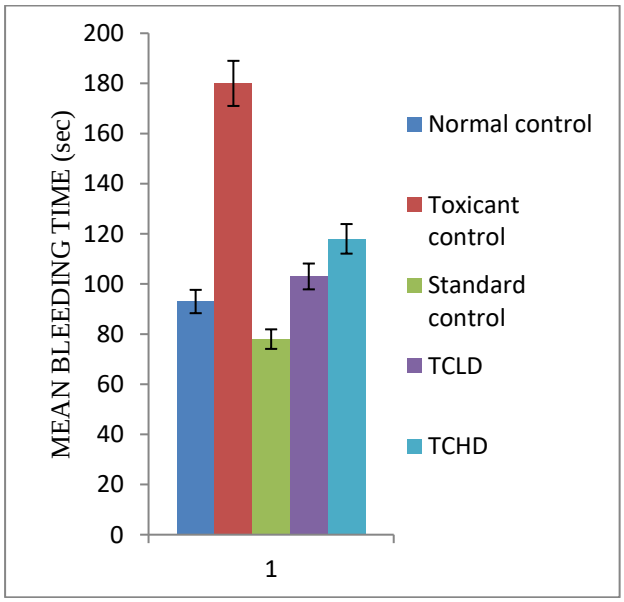

Figure 2: Graphical representation of mean bleeding time (sec) (Model 1)

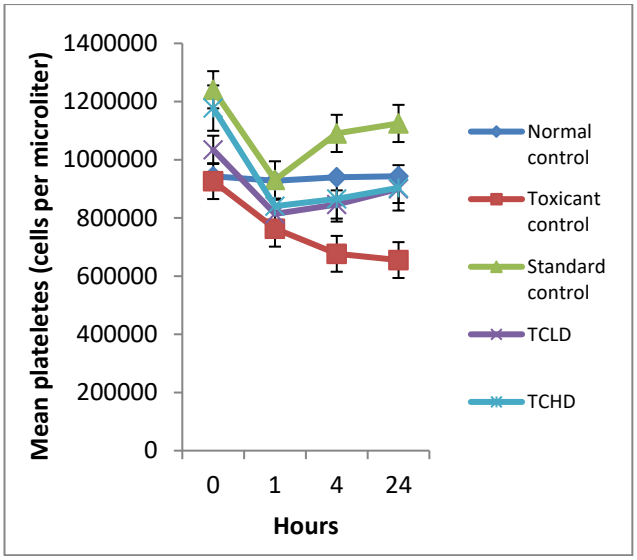

Figure 3: Graphical representation of mean platelet count with hours (Model 2)

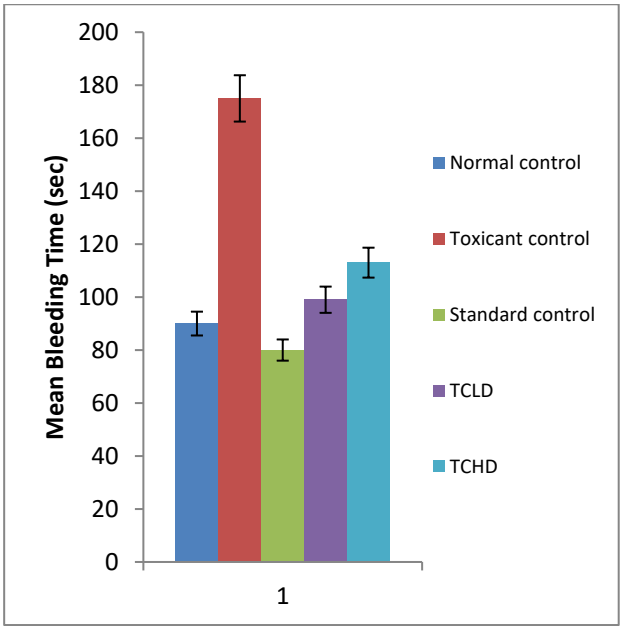

Figure 4: Graphical representation of mean bleeding time (sec) (Model 2)

DISCUSSION

The hydroalcoholic leave extract of *Tinospora cordifolia*, significantly shows thrombocytogenic and, thrombocytoprotective activity may be due to its previously established antioxidant [5], antimicrobial [8] and, immunomodulatory effect [11]. Lipid per oxidation is an important mechanism for any type of cellular damage. Any drug which can show free radical scavenging activity obviously shows a certain level of protection towards cells and tissues.

Antimicrobial, immunomodulatory are the major two activities to protect and boost our body defence by encouraging the formation of new cells and the elimination of derbies. The above described any/all of this property may be responsible for the thrombocytogenic activity of *Tinospora cordifolia.*

CONCLUSION

The present study was undertaken to evaluate the form of thrombocytogenic activity of Tinospora cordifolia. The observation supports it's used in the treatment of dengue fever or to heal the open cut. Further detailed study on the same topic will be encouraged to come out with the exact molecular mechanism of Tinospora cordifolia as a thrombocytogenic agent.

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