



Evaluation of Role of *Tinospora Cordifolia* (T.cord.) In Experimentally Induced (Busulfan Induced) Thrombocytopenia In Rabbits

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ABSTRACT

Introduction: *Tinosporacordifolia* has been used in the indigenous system of medicine since the Vedic period and considered as one of the best drugs in terms of availability, economy and ease of administration. **Aim & objective:** To evaluate the role of *tinospoacordifolia* in experimentally induced thrombocytopenia in rabbits. **Materials & methods:** The study was conducted in conscious albino rabbits of either sex weighing 1.5-2.5kg, divided into 7 groups with 6 rabbits in each. Platelet counts before drug administration served as its own control. Group 1 was given normal saline 5ml/kg P/O, group 2 busulfan 50mg/kg P/O in a single dose once only, group 3 aq. ext. of *T. Cord.* 4ml/kg P/O and group 4 & 5 were given aq. ext. of *T. Cord.* 2ml/kg & 4ml/kg respectively started on 14th day (after attaining maximum thrombocytopenia by busulfan). Blood was withdrawn on day 1 and then weekly i.e. on 7th, 14th, 21st, 28th, 35th & 42nd days of study for the study of platelet counts by preparing peripheral blood smear. **Results-** Gr. IV & V showed a significant improvement in platelet counts. This beneficial effect of aq. ext. of *T. Cord.* suggests that it probably acts by improving the production of platelets from bone marrow due to its antioxidant and immunomodulatory properties. This beneficial role of *T. Cord.* can be attributed to the presence of various phytochemical constituents like alkaloids, sesquiterpenoid and glycosides present in the stem. **Conclusion:** Thus it was concluded from our study that the aqueous extract possesses therapeutic role in thrombocytopenia.

KEYWORDS

TinosporaCordifolia, Thrombocytopenia, Busulfan, Dengue

Introduction:

Thrombocytopenia is a disorder in which there is an abnormally low amount of platelets. Platelets play an important role in clotting and bleeding. So, this condition is sometimes associated with abnormal bleeding.¹ Normal platelet count ranges between 1.5 lakh/mm³ to 4.5 lakh/mm³ of blood. Lesser than 1.5 lakh/mm³ is termed as thrombocytopenia whereas greater than 4.5 lakh/mm³ is termed as thrombocytosis.¹ Thrombocytopenia may occur due to decrease synthesis or increase breakdown of the platelets in blood stream or in the spleen or liver.^{4,5,6,7,8,9,10} There are certain drugs like Busulfan, Valproic acid, Methotrexate, Carboplatin, Interferon etc. that produce direct myelosuppression effect thus inducing thrombocytopenia.¹¹ Some other causes of thrombocytopenia includes the common viral infections like dengue, rubella, mumps etc.² which are found to be associated with thrombocytopenia among them dengue is currently regarded globally as the most important mosquito-borne viral disease, which proves to

be fatal due to severe bleeding from various sites of the body and for which there is no specific treatment.^{2,3}

The treatment modalities to increase the platelet count include the use of corticosteroids & Lithium carbonate having their own serious side effects.^{12,13} Another modality is Platelet Infusion to increase the platelet count, but it has minimal effect in autoimmune thrombocytopenia (e.g. – Hemolytic Uremic Syndrome, Thrombotic Thrombocytopenic Purpura).¹⁴ All these treatment modalities are costlier and increase the hospital stay and also associated with increased risk of mortality and morbidity.

Some indigenous drugs like "*TinosporaCordifolia*", "*TriticumAestivum*" and "*Boerhaaviadiffusa* Linn" etc. are claimed by Ayurvedacharyas to have a large potential to increase the platelet counts^{15,16} and are frequently used as aqueous extract by the patients suffering from dengue on their own as

a home remedy. At the same time they are also claimed to be free from side effects but there is little substantial evidence to prove this experimentally. So the present study has been planned to elucidate the effect of aqueous extract of the stem of "TinosporaCordifolia (Giloe)" in experimentally induced thrombocytopenia in rabbits.

Objective: The objective of this study was to evaluate the role of Tinosporacordifolia (T.cord.) in experimentally induced (busulfan induced) thrombocytopenia in rabbits.

Materials & methods: The present study was conducted on adult rabbits (1.5-2.5 kg) of either sex after the approval from institutional animal ethical committee (IAEC) according to the guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA). The animals were acclimatized to laboratory condition for 15 days before commencement of experiment and maintained under standard laboratory condition (light period of 12 h/day and at room temperature), with access to commercial pellet diet and water ad libitum. The food was withdrawn 12 hours before and during the experimental hours. A minimum of six animals were taken in each group and were divided into five groups :

Group I was treated with saline (5ml/kg orally) for a period of 6 weeks and served as control to assess the spontaneous change in platelet count.

Group II (thrombocytopenic group) was challenged with Busulfan (50 mg/kg orally) single dose on 1st day of the study i.e. once only for the development of thrombocytopenia; ¹⁷

Group III was given aqueous extract of T. Cord. (aq. Ext. of T. Cord.) (4ml/kg orally) twice daily for a period of 6 weeks for perse effect;

Group IV & V were challenged with Busulfan (50 mg/kg orally) single dose only on 1st day of study and treatment with 2ml & 4ml/kg aq. ext. of T. cord. orally twice daily was started after 14 days of busulfan administration (when the maximum

thrombocytopenia developed) to study the therapeutic effect of the test drug.

Blood was withdrawn from marginal vein of ear on day 1 and then weekly i.e. on 7th, 14th, 21st, 28th, 35th & 42nd days of study for the study of platelet counts and the blood was collected in EDTA tube to prevent clot formation. Then platelet count was done by preparing peripheral blood smears ¹⁸.

The results were statistically analysed using the unpaired Student's t test and presented as mean ± SD. Group (gr.) II and gr. III were compared with gr. I and gr. IV and V were compared with group II. P values were calculated referring to appropriate tables. Values of P<0.05 were considered as statistically significant.

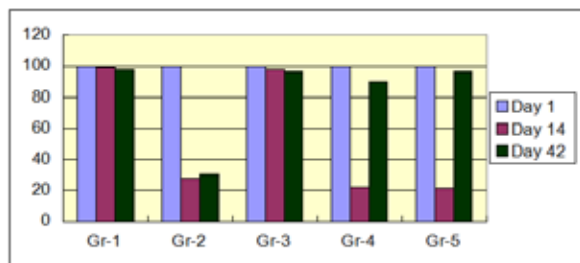
Results: Rabbits of saline treated gr. I and only T.Cord. treated gr. III did not show any significant variation in platelet counts during the period of six weeks of study [table1&fig1], while the animals of gr. II which were challenged with busulfan (50mg/kg orally) single dose once only on first day of study, showed significant reduction in platelet counts starting from the first week with a maximum decrease in platelet count on day 14th (0.92±0.10 lakh/mm³ i.e. 72.35±4.99% decrease) and remained significantly low till sixth week (-69.43±5.95% decrease)(table1&fig1). While In Gr. IV and V, which were challenged with aq. ext. of T. Cord. in the doses of 2ml and 4ml/kg orally twice daily respectively for assessing the curative effect after the development of thrombocytopenia, the platelet counts started increasing significantly with maximum rise seen in first week after starting the treatment with aq. ext. of T. Cord. from 0.84±0.10 & 0.8±0.10 lakh/mm³ resp. on day 14 (i.e. 77.73±3.53% & 78.27±2.47% dec.) to 1.86±0.17 & 1.97±0.11 lakh/mm³ resp. on day 21 (0.65±6.42% & 46.47±4.41% dec.) and it continued to increase gradually till 6th week of the study (3.39±0.18 & 3.56±0.16 lakh/mm³ on day 42 i.e. 10.19±8.90% & 3.39±6.58% dec. resp.) [table1&fig1]. The therapeutic effect in gr. IV & V, as shown by the graded doses of test drug when compared with busulfan induced thrombocytopenic gr. II showed a significant improvement in platelet counts.

Table-1
Effect of administration of Aq. Ext. of T. Cord. Per se (Gr. III) and 2ml & 4ml/kg of T. Cord. Twice daily from 14th day to 42nd day on busulfan (50mg/kg once only) induced thrombocytopenia and its comparison with Gr. II. The initial platelet count was considered to be 100%.

DRUGS	Percent change in Platelet count (Mean ± SD)						
	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
N.S. 5ml/kg orally (Group I)	100% 3.65lakh	-0.9±2.78 3.62lakh	-1.01±2.26 3.61lakh	-2.42±3.14 3.56lakh	1.05±1.04 3.61lakh	0.04±2.88 3.66lakh	2.06±7.43 3.73lakh
Busulfan 50mg/kg orally (Group II)	100% 3.33lakh	-69.67±6.36### 1.01lakh	-72.35±4.99### 0.92lakh	-70.61±4.98### 0.98lakh	-69.64±5.39### 1.01lakh	-68.91±5.01### 1.04lakh	-69.43±5.95### 1.02lakh
Aq. ext. of T. Cord. 4ml/kg orally twice daily (Group III)	100% 3.69lakh	0.69±4.42 3.72lakh	1.94±3.01 3.76lakh	1.20±4.19 3.73lakh	2.08±2.55 3.77lakh	0.22±3.56 3.70lakh	3.23±5.26 3.81lakh
Busulfan 50mg/kg orally on day 1 only & Aq. ext. of T. Cord. 2ml/kg orally twice daily from day 14 to day 42 (Group IV)	100% 3.77lakh	-78.21±4.07 0.82lakh	-77.73±3.53 0.84lakh	-50.65±6.42*** 1.86lakh	-36.01±9.07*** 2.41lakh	-20.99±9.73*** 2.98lakh	-10.19±8.90*** 3.39lakh
Busulfan 50mg/kg orally on day 1 only & Aq. ext. of T. Cord. 4ml/kg orally twice daily from day 14 to day 42 (Group V)	100% 3.68lakh	-78.39±4.12 0.79lakh	-78.27±2.47 0.80lakh	-46.47±4.41*** 1.97lakh	-32.17±6.44*** 2.50lakh	-12.64±6.23*** 3.21lakh	-3.39±6.58*** 3.56lakh

- in comparison to saline, * - in comparison to Busulfan only, # p<0.05 (Significant), ## p<0.01 or ### p<0.001 (Highly Significant), * <0.05 (Significant), **<0.01 or ***<0.001 (Highly Significant), n =6, degree of freedom-10, confidence interval-95%

Fig-1
Effect of Aq. Ext. of T. Cord. (2ml & 4ml/kg orally twice daily for 6 weeks) on Busulfan (50mg/kg orally once only) induced thrombocytopenia (depicted as percent change) as a curative measure



Discussion: Thrombocytopenia is a condition in which platelet count gets lower than normal number of platelets in the blood. Thrombocytopenia can be associated with infectious diseases, hereditary conditions, autoimmune diseases as well as drug induced (e.g.-busulfan, valproic acid, isotretinoin, cyclophosphamide, panobinostat etc.)^{19,20,21}. Dengue is one of the important causes of thrombocytopenia. Dengue virus induces bone marrow suppression.²² Since bone marrow is the manufacturing center of blood cells, its suppression causes deficiency of blood cells leading to low platelet count (thrombocytopenia), leucocytopenia, anaemia and spontaneous severe bleeding are the other consequences of bone marrow suppression. Dengue virus can bind to human platelets in presence of virus specific antibody and cause immune mediated clearance of platelets²³. The use of T.Cord. in Dengue has been advocated by many Ayurvedacharyas and many Ayurvedic literatures have quoted its use in dyspepsia, fever, pyrexia and immunomodulation.^{24,25} This suggests that T. Cord. may possess an improvising role in platelet production and decreasing platelet destruction. In our study, for evaluation of beneficial effects of aq. ext. of T. cord. in thrombocytopenia, busulfan was used to induce experimental thrombocytopenia.²⁶ A significant thrombocytopenia was evident in gr. II over the period of six weeks with a maximum decrease in 2nd week (table 1 & fig.1) but treatment with fresh aq. ext. of T. cord. in graded doses (2 & 4 ml/kg orally twice in a day) after producing thrombocytopenia i.e. after two weeks, showed significant increase in platelet counts in all rabbits of gr. IV & V as compared to busulfan treated gr. II (table 1 & fig.1). Busulfan is an alkylating agent with myeloablative activity i.e. bone marrow suppression²⁷ and immune suppression²⁸ properties and activity against non-dividing marrow cells and possibly, non-dividing malignant cells.²⁶ The beneficial effect of aq. ext. of T. Cord. in busulfan induced thrombocytopenia, suggests that it probably acts by improving the production of platelets from bone marrow due to its antioxidant²⁹ and immunomodulatory properties³⁰. This beneficial role of T. Cord. can be attributed to the presence of various phytochemical constituents like alkaloids (berberine, palmatine D, choline D, tinosporine, magnoflorine, tetrahydropalmatine), sesquiterpenoid (tinocordifolin) and glycosides (furanoid diterpene glycoside, tinocordiside, syringing and syringine-apiosylglycoside) present in the stem.³¹ Alkaloids present in stem of T.Cord. have been proven effective against anti-platelet macrophages in patients suffering from Idiopathic Thrombocytopenic Purpura (ITP)³² indicating the role of T. Cord. in protecting the platelets from destruction. The clinical studies have shown that T.Cord. also possesses hepatoprotective³³, antipyretic³⁴ and immunomodulatory³⁵ activities and it has also been said to be beneficial in Post-menopausal syndrome.³⁶ The drug is considered to be safe with no significant information on side effects is available so far except mild nausea in humans due to its bitter taste. However the exact mechanism of action for T. Cord. can not be ascertained and further studies are required.

Conclusion- Administration of Giloe i.e. T.Cord. increased the platelet counts significantly in Busulfan induced thrombocytopenic rabbits. So, T.Cord. is beneficial & cost-effective in thrombocytopenia and with no side effects. In future, its use along

with the drugs causing thrombocytopenia as a side effect may also be considered.

References:

1. Thrombocytopenia <http://en.wikipedia.org/wiki/Thrombocytopenia>, Accessed on 10th December, 2014
2. Bhatt S, Gething PW, Brady OJ, et al. (April 2013). "The global distribution and burden of dengue". *Nature* 496 (7446): 504-7.
3. Gubler DJ. Dengue/dengue haemorrhagic fever: history and current status. *Novartis Found Symp.* 2006;277:3-16.
4. Maton, Anthea; Jean Hopkins, Charles William McLaughlin, Susan Johnson, Maryanna Quon Warner, David LaHart, Jill D. Wright (1993). *Human Biology and Health*. Englewood Cliffs, New Jersey, USA.
5. Konkle BA. Disorders of platelets and vessel wall. In: *Harrison's Principles of Internal Medicine*. Vol. 1, 17th edition, Fauci AS, Braunwald E, Kasper DL, et al. (Eds.), McGraw-Hill: New York, NY 2008:p.718-23.
6. Craig JIO, McClelland DBL, Watson HG. Thrombocytopenia. In: *Davidson's Principles and Practice of Medicine*. 21st edition, College NR, Walker BR, Ralston SH (Eds.), Churchill Livingstone Elsevier: Edinburgh 2010:p.1003-4.
7. Bichile SK. Platelet disorder. In: *API Textbook of Medicine*. Vol. 1, 9th edition, Munjal YP (Ed.), Jaypee Brothers: New Delhi 2012:p.987-8.
8. Firkin F, Penington D, Chesterman C, Rush B. The hemorrhagic disorders. In: *deGruchy's Clinical Hematology in Medical Practice*. 5th edition, Oxford University Press: Bombay 1989:p.375-92.
9. Lee GR, Foerster J, Lukens J, Paraskevas F, Greer JP, Rodgers GM. Shirley Parker Levine - Thrombocytopenia: Pathophysiology and Classification. In: *Wintrobe's Clinical Haematology*. Vol. 2, 10th edition, Lipincott Williams & Wilkins: Philadelphia 1999:p.1579-82.
10. Diz-Kucukkaya R, Chen J, Geddis A, Lopez JA. Thrombocytopenia. In: *William's Hematology*. 8th edition, Kaushansky K, Lichtman MA, Beutler E, Kipps TJ, Selisohn U, Prchal JT, (Eds.) New York 2011:p.1891-918.
11. Greslele, Paolo; Fuster, Valentin; Lopez, Jose A.; Page, Clive P.; Vermylen, Jos (2007-12-13). Platelets in Hematologic and Cardiovascular Disorders: A Clinical Handbook. Cambridge University Press. ISBN 9781139468763. Retrieved 2015-04-30.
12. Corticosteroids <http://en.wikipedia.org/wiki/Corticosteroid>, Accessed on 10th December, 2014
13. Simard, M; Gumbiner, B; Lee, A; Lewis, H; Norman, D (1989). "Lithium carbonate intoxication. A case report and review of the literature". *Archives of Internal Medicine* 149 (1): 36-46
14. Plateletpheresis <http://en.wikipedia.org/wiki/Plateletpheresis>, Accessed on 10th December, 2014
15. Bharati P, Sinha R, Study The Effect of Tinospora Cardifolia (Wild) Miers and Boerhaavia Diffusia Linn On Dengue, *International Journal of Ayurvedic and Herbal Medicine* 2012; 2 (3)
16. P. R. Tirgar, INVESTIGATION INTO THERAPEUTIC ROLE OF TRITICUM AESTIVUM (WHEAT) GRASS IN BUSULFAN INDUCE THROMBOCYTOPENIA, *International Journal of Universal Pharmacy and Life Sciences* 2011; 1 (1)
17. Evensen SA, Jeremic M, Hjort PF: Experimental thrombocytopenia induced by busulphan (Myleran) in rabbits: extremely low platelets levels and intact plasma clotting system. *ThrombDiathHaemorrh* 19:570, 1968
18. Jain NC. *Schalm's veterinary hematology*, 4th ed. Philadelphia: Lea and Febiger, 1986.
19. Visentin GP, Liu CY. Drug-induced thrombocytopenia. *HematolOncolClin-North Am.* 2007;21:685-696, vi.
20. Aster RH, Bougie DW. Drug-induced immune thrombocytopenia. *N Engl J Med.* 2007;357:580-587.
21. Rizvi MA, Kojouri K, George JN. Drug-induced thrombocytopenia: an up-dated systematic review. *Ann Intern Med.* 2001;134:346
22. Gubler J, Dengue and Dengue Hemorrhagic Fever. *ClinMicrobiol Rev* 1998; 11(3):480-496.
23. Wang J, Xu J, Zhong J, Effect of Radix notoginseng saponins on platelet activating molecule expression and aggregation in patient with blood hyperviscosity syndrome. *ZhinggguoZhong Xi Yi Jie He ZaZhi* 2004; 24(4):312-316
24. Krishnamurti C, Peat R, Cutting M, Rothwell S, Platelet adhesion to Dengue-2 virus infected cell. *American Journal of Tropical medicine* 2002; 66(4):435-441.
25. PriyankBharati. Study the effect of Tinospora Cardifolia Miers and Boerhaavia diffusa Linn on dengue. *International Journal of Ayurvedic and Herbal Medicine*, 2012; Vol. 2(3): 574-577
26. Brahmavarchas, *Ayurvedka PranVanoshadi Vigyan*, 6th edition, YugNirman Yojna Gayatri Tapobhumi, Mathura, 2006, 100-101
27. Evensen SA, Jeremic M, Hjort PF: Experimental thrombocytopenia induced by busulphan (Myleran) in rabbits: extremely low platelets levels and intact plasma clotting system. *ThrombDiathHaemorrh* 19:570, 1968
28. Yeager AM, Shinn C, Shinohara M, Pardoll DM (1993) Hematopoietic stem

- cell transplantation in the twitcher mouse. The effects of pretransplant conditioning with graded doses of busulfan. *Transplantation* 56(1): 185–90.
29. Eberly, AL.; Anderson, GD.; Bubalo, JS.; McCune, JS. (Dec 2008). "Optimal prevention of seizures induced by high-dose busulfan." *Pharmacotherapy* 28 (12): 1502–10
 30. Barik Rakesh Sanjay Jain, Bhumika Sherlekar. Evaluation of Antioxidant Potential of *Tinospora Cordifolia* and *Tinospora Sinensis*. *IJPSR*, 2010; Vol. 1 (11): 122-128.
 31. Kapil A, Sharma S. Immunopotentiating compounds From *Tinospora cordifolia*. *J Ethnopharmacol.* 1997;58:89-95
 32. Chintalwar G, Jain A, Sipahimalani A, Banerji A, Sumariwalla P, Ramakrishnan R and Sainis K *Phytochemistry* 1999;52:1089–1093.
 33. S Gupta KC, Viswanathan R. Antituberculous substances from plants. *Antibiot & Chemother* 1956; 6:194-5.
 34. Jeyachandran R, Xavier TF, Anand SP. Antibacterial activity of Stem extract of *Tinospora cordifolia*. *AncSci Life* .2003;23:40-43.
 35. Kavitha B. T, Ramachandra L Y, S. D. Shruthi, S. Padmalatha Rai. Phytochemical analysis and hepatoprotective properties of *Tinospora cordifolia* against carbon tetrachloride-induced hepatic damage in rats. *Journal of Basic and Clinical Pharmacy*. 2011:139-142.
 36. Mehrotra R, Katiyar CK, Gupta AP. Hepatoprotective compositions and composition for treatment of conditions related to hepatitis B and E infection. US Patent 749296.2000.