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

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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. Normal platelet count ranges between 1.5 lakh/mm$^3$ to 4.5 lakh/mm$^3$ of blood. Lesser than 1.5 lakh/mm$^3$ is termed as thrombocytopenia whereas greater than 4.5 lakh/mm$^3$ is termed as thrombocytosis. Thrombocytopenia may occur due to decrease synthesis or increase breakdown of the platelets in blood stream or in the spleen or liver. There are certain drugs like Busulfan, Valproic acid, Methotrexate, Carboplatin, Interferon etc. that produce direct myelosuppression effect thus inducing thrombocytopenia. Some other causes of thrombocytopenia includes the common viral infections like dengue, rubella, mumps etc. 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.

The treatment modalities to increase the platelet count include the use of corticosteroids & Lithium carbonate having their own serious side effects. 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). All these treatment modalities are costly and increase the hospital stay and also associated with increased risk of mortality and morbidity.

Some indigenous drugs like “Tinospora Cordifolia”, “Triticum Aestivum” and “Boerhaavia diffusa Linn” etc. are claimed by Ayurvedacharyas to have a large potential to increase the platelet counts 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 “Tinospora Cordifolia (Giloe)” in experimentally induced thrombocytopenia in rabbits.

**Objective:** The objective of this study was to evaluate the role of Tinospora cordifolia (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 (IAECC) 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 per se effect;
- **Group IV & V** were challenged with Busulfan (50 mg/kg orally) twice daily for a period of 6 weeks for the graded doses of test drug when compared with busulfan induced thrombocytopenic gr. II showed a significant improvement in platelet counts.

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- **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 per se effect;
- **Group IV & V** were challenged with Busulfan (50 mg/kg orally) single dose 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 14 (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 acq. 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 acq. ext. of T. Cord. from 0.84±0.10 & 0.8±0.10 lakh/mm³ resp. on day 14 (i.e. 77.73±5.33% & 78.27±4.47% dec.) to 1.86±0.17 & 1.97±0.11 lakh/mm³ resp. on day 21 (50.65±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%.

<table>
<thead>
<tr>
<th>DRUGS</th>
<th>Percent change in Platelet count (Mean ± SD)</th>
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<tbody>
<tr>
<td></td>
<td>Day 1</td>
</tr>
<tr>
<td>N.S. 5ml/kg orally (Gr. II)</td>
<td>100%</td>
</tr>
<tr>
<td>Busulfan 50mg/kg orally (Gr. II)</td>
<td>100%</td>
</tr>
<tr>
<td>Aq. ext. of T. Cord. 4ml/kg orally twice daily (Gr. III)</td>
<td>100%</td>
</tr>
<tr>
<td>Busulfan 50mg/kg orally on day 10only &amp; Aq. ext. of T. Cord. 2ml/kg orally twice daily from day 14 to day 42 (Gr. IV)</td>
<td>100%</td>
</tr>
<tr>
<td>Busulfan 50mg/kg orally on day 10only &amp; Aq. ext. of T. Cord. 4ml/kg orally twice daily (Gr. V)</td>
<td>100%</td>
</tr>
</tbody>
</table>

* - 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), leukocytopenia, anaemia and spontaneous severe bleed 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 suppression 27 and immune suppression 28 properties and activity against non-dividing marrow cells and possibly, non-dividing malignant cells.29 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 antioxidant 29 and immunomodulatory properties.30 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, tetrahydroplatinatine), sesquiterpenoid (tino-cordifolin) and glycosides (furanoidditerpene glycoside, tinosporine), like alkaloids (berberine, palmatine D, choline D, tinosporine, cordifolin) and glycosides (furanoidditerpene glycoside, tinosporine-apiosyglycoside) present in the stem.31 Alkaloids present in stem of T.Cord. have been ascertained and further studies are required.

Conclusion- Administration of Giloe i.e. T.Cord. increased the platelet counts significantly in Busulfan induced thrombocyto- penic 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.

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