Ameliorative Potential of Sap of Borassus Flabellifer Against Cyclophosphamide Induced Myelotoxicity In Wistar Rats

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Objective: The present study was undertaken for investigating the ameliorative potential of sap of Borassus flabellifer against cyclophosphamide induced myelotoxicity in wistar rats. Materials & Methods: The present experimental study was conducted on various groups of animals each with six healthy adult rats. Hematological parameters i.e., RBC count, total hemoglobin, packed cell volume, MCV, WBC count, total platelets and differential leucocyte counts were determined in various groups of animals. Results: The treatment with sap of Borassus flabellifer at dose 3.6 ml/kg significantly elevates RBC, hemoglobin and white blood cells of blood. This observation suggests that Borassus flabellifer sap defend the bone marrow from adverse reactions of cyclophosphamide in rats. Conclusion: In conclusion, the present study suggests that, treatment with Borassus flabellifer sap at dose of 3.6 ml/kg have significant protective effect against cyclophosphamide induced myelosuppression in wistar rats.

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INTRODUCTION:

Cancer is a genetic disease, caused by changes to genes that regulate the functions of cell growth and division. There are many types of cancer treatments such as radiation therapy, surgery and chemotherapy \[1\]. Chemotherapy is most effective at killing cancer cells but causes side effects as well such as myelosuppression, lung injury, nephrotoxicity, & cardiotoxicity \[2\].

Cyclophosphamide (CTX) is a potent anticancer drug, effective against malignant and non-malignant disorders. The usage of cyclophosphamide is limited because of its widespread adverse effects due to generation of reactive oxygen species (ROS) that react with biomolecules to yield secondary radicals that often induce chain reactions. ROS reacts with biomolecules which results in cross linking of protein, DNA adduct which are detrimental to the functioning of cell, leading to toxicities \[3\]. The toxic metabolites of CTX, acrolein & phosphoramidate mustard are responsible for various organ toxicities with a major effect on bone marrow causing myelosuppression, immunosuppression, bone marrow failure and infections \[4, 5, 6\].

Several new strategies are being developed to control and treat cyclophosphamide induced myelosuppression. One such approach is a combination of an antioxidant with chemotherapeutic agents, which enhance efficacy while reducing toxicity to normal tissues \[7, 8\]. The antioxidants may minimize the toxic side effects of chemotherapeutic agents and may supply the use of more effective high doses of the anticancer drugs \[9\].

Herbal medicines have been used throughout history and within every culture to prevent and treat diseases. *Borassus flabellifer* L., belongs to family Arecaceae, commonly known as palmyra palm, is a native of tropical Africa but cultivated and naturalized throughout India, along the coastal areas of Tamil Nadu, Kerala and Bengal \[10\]. Modern science has proved that fruits, leaves, bark, roots, sap of palmyra palm play disease preventing benefits. Plant considered a rich source of phytoconstituents gums, saponins, glycosides, carbohydrates, fats, vitamins A, B, and C. The dietary nutrients have beneficial antioxidants which can delete harmful active oxygen species, such as O₂, H₂O₂ and OH \[11\]. *B. flabellifer* exhibits anti-inflammatory, antibacterial, analgesic, cytotoxic, antipyretic, hypoglycemic, antioxidant antifungal and antihelminthic \[12, 13, 14\].

Sap of *B. flabellifer* exudates from the phloem tissue and has a crucial role in Ayurvedic medication. Fresh unfermented sap contains sugar, proteins, lipids, amino acids, steroids, vitamins & minerals \[15\]. Sap from the flower stalk is used as a tonic, diuretic, stimulant, laxative and antiphlegmatic and amoebicide. It is a household remedy for many ailments such as cholera, indigestion, constipation, piles, insomnia & hepatitis \[16\].
Several organic acids such as citric acid, tartaric acid, malic acid, lactic acid, fumalic acid, pyrogallic acid and high content of succinic acid had been reported which have antioxidant activities. Currently, evidence is growing that antioxidants may provide benefit when combined with certain types of chemotherapy [17]. The amount of nutrients, minerals, vitamins and organic acids present in 100 ml fresh sap is given below,

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Quantity</th>
<th>Parameters</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Sugars (g)</td>
<td>7.20</td>
<td>Retinol (IU)</td>
<td>74</td>
</tr>
<tr>
<td>Total Proteins (g)</td>
<td>19.70</td>
<td>Ascorbic Acid (mg)</td>
<td>4.95</td>
</tr>
<tr>
<td>Total Lipids (g)</td>
<td>0.08</td>
<td>Niacin (mg)</td>
<td>0.48</td>
</tr>
<tr>
<td>Magnesium (mg)</td>
<td>5.61</td>
<td>Thiamin (mg)</td>
<td>0.06</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>5.21</td>
<td>Riboflavin (mg)</td>
<td>0.04</td>
</tr>
<tr>
<td>Phosphorus (mg)</td>
<td>1.85</td>
<td>Succinic Acid (mg)</td>
<td>34</td>
</tr>
<tr>
<td>Potassium (mg)</td>
<td>1.91</td>
<td>Malic Acid (mg)</td>
<td>15</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>1.12</td>
<td>Lactic Acid (mg)</td>
<td>9</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>1.50</td>
<td>Citric Acid (mg)</td>
<td>0.9</td>
</tr>
<tr>
<td>Copper (mg)</td>
<td>0.06</td>
<td>Pyroglutamic Acid (mg)</td>
<td>0.9</td>
</tr>
<tr>
<td>Zinc (mg)</td>
<td>0.04</td>
<td>Tartaric Acid (mg)</td>
<td>0.6</td>
</tr>
</tbody>
</table>

The present study was carried out to screen the myeloprotective effect of sap of *Borassus flabellifer*, in cyclophosphamide induced myelotoxicity in wistar rats.

**MATERIAL AND METHODS**

**Drug Material**

Cyclophosphamide (Phosmid) was acquired commercially from NEON Laboratory Ltd., Mumbai. CTX (50 mg) was dissolved in distilled water to appropriate concentration prior to the injection. Single dose of CTX (150 mg/kg b.w.) was used intraperitoneally.

**Chemicals and Drugs**

Gower’s solution & WBD diluting fluid were obtained from S.D. Fine Chemicals Ltd., Mumbai. Drabkin’s solution used to measure hemoglobin concentration obtained from ARKRAY Healthcare Pvt. Ltd., platelet diluting fluid from Bio Lab Diagnostics Pvt. Ltd., Mumbai and Leishman’s stain from Nice Chemicals Pvt. Ltd., Cochin.
Collection of Sap
The sap of *B. flabellifer* was collected from the village of Jalpally, Ranga Reddy District in the month of April, 2019. It was collected early in the morning before sunrise and preserved in chilled condition at temperature below 0° C to prevent microbial degradation of chemical constituents. The sap was transported to the research laboratory and the purity was assessed by its physical properties. The colour, odour, taste and pH were evaluated for differentiating sap from fermentation on the basis of literature description [18, 19].

Preliminary Phytochemical Analysis
The sap of *B. flabellifer* was screened for the following phytochemical principles; alkaloids, carbohydrates, saponins, glycosides, proteins and amino acids, flavonoids, phytosterols and phenols using simple established methods as outlined by Gusthinnadura et al., 2017 [20].

Experimental Animals
All the experimental procedures were carried out in accordance with Committee for the Purpose of Control and Supervision of Experiments on Animal (320/CPCSCEA dated 03-01-2001) guidelines. The study was approved by Institute’s Animal Ethical Committee and confirmed to national guidelines on the consideration and utilization of research facility animals (GPRCP/IAEC/23/19/02/PCL/AE-2-Rats-M/F-24).

Albino wistar rats of either sex (200-250gm) were obtained from Sainath Agencies, Uppal, Hyderabad, India. They were housed individually in an environmentally controlled room with 12-h light/dark cycle and had free access to food and water. After a seven days acclimatization period, they were randomly selected for different experimental groups.

Selection of Cyclophosphamide Dose
Selection of the cyclophosphamide dose of 150 mg/kg b.w. for inducing myelosuppression reliable with literature data, recommending a single administration of 100 mg/kg b.w. [21], 150 mg/kg b.w. [9], or 200 mg/kg b.w. [7]. Hence, myelosuppression was induced in animals by administrating cyclophosphamide with a single dose of 150 mg/kg intraperitoneally once on 6th day of experiment, followed by administration of test substance for remaining 4 days.

Selection of Sap Dose
The dose of sap of *B. flabellifer* was selected based on the previous literature data from direct human studies [15]. Dose for animal was extrapolated from human dose. No morbidity was observed during and after completion of the study.

Experimental Design
This study was intended for 10 days, using albino wistar rats weighing 200-250 gm of either sex. Experimental animals were randomly divided into 4 groups, each group containing 6 animals.

- **Group 1 (Normal Control):** Animals received normal saline, 1ml/kg, p. o.
- **Group 2 (Sap Control):** Animals received sap of *Borassus flabellifer*, 3.6 ml/kg b. w., p. o.
- **Group 3 (Disease Control):** Animals received CTX, 150 mg/kg, i. p., on 6th day
- **Group 4 (Treatment Control):** Animals received sap of *Borassus flabellifer*, 3.6 ml/kg b. w., p. o. and CTX, 150 mg/kg, i. p., on 6th day.

On initial (0th) and final (11th) day, the blood was collected from each rat by retro-orbital plexus and markers of myelosuppression was evaluated by hematological parameters i.e., red blood cell (RBC) count, hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), total white blood cell (WBC) count, platelets and differential leucocyte counts (DLC).

Hematological Assessment
Blood samples from experimental animals were collected into EDTA (ethylenediamine-tetraacetaete) bottles and analyzed using standard procedures. RBC & WBC count were performed according to the method describe by Cheesbrough & McArthur, 1976 [22]. Hb was measured by spectrophotometric method explained by Drabkin & Harold, 1932 [23]. PCV obtained by the method described by Gitte, 2004 using centrifuge machine [24]. DLC was performed according to Blumenreich, 1990 [25] and platelets were counted according to the procedure of Brecher et al., 1953 [26].

Statistical Analysis
Data were analyzed with a statistical software (Graphpad Prism 7) and values were expressed as Mean ± SEM and differences between the groups were
statistically determined by analysis of variance (ANOVA) followed by Tukey’s Multiple Comparison test. Statistically significant levels were considered at \( P <0.0001 \) as extremely significant, \( P <0.01 \) moderately significant, \( P <0.05 \) significant.

**RESULTS**

**Preliminary Phytochemical Screening**
The preliminary phytochemical screening of sap of *Borassus flabellifer* showed the presence of alkaloids, carbohydrates, saponins, proteins and amino acids.

**Effect of Sap of *Borassus flabellifer* on Hematological Parameters**
The results of hematological parameters were given in table 1. A significant decrease in red blood cells, hemoglobin, packed cell volume, mean corpuscular volume and platelets was observed in cyclophosphamide treated animals compared to their baseline values measured on initial day. It is indicative of myelosuppressive anemia and thrombocytopenia. However, these myelosuppressive changes in hematology returned to normal in animals treated with *B. flabellifer* sap. There was no meaningful change in hematological parameters in animals of normal control group and sap control group which received sap alone. The animals showed evident inhibitory effects on cyclophosphamide-induced myelosuppressive anemia and thrombocytopenia, due to *B. flabellifer* sap treatment.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatment</th>
<th>Normal (Saline, 1ml/kg)</th>
<th>Sap (Palmyra Sap, 3.6 ml/kg)</th>
<th>Disease (CTX, 150mg/kg)</th>
<th>Treatment (Palmyra Sap 3.6 ml/kg + CTX 150 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>11</td>
<td>0</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>RBC (million/mm(^3))</td>
<td>8.18 ± 0.28</td>
<td>8.72 ± 0.32</td>
<td>8.72 ± 0.18</td>
<td>9.17 ± 0.27</td>
<td>8.05 ± 0.17</td>
</tr>
<tr>
<td>Hb (mg/dl)</td>
<td>13.78 ± 0.24</td>
<td>13.83 ± 0.12</td>
<td>14.98 ± 0.28</td>
<td>15.50 ± 0.29(^b)</td>
<td>13.95 ± 0.28</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>43.63 ± 0.32</td>
<td>43.48 ± 0.34</td>
<td>44.90 ± 0.34</td>
<td>46.12 ± 0.82</td>
<td>43.40 ± 0.30</td>
</tr>
<tr>
<td>MCV (%)</td>
<td>53.66 ± 1.90</td>
<td>52.96 ± 2.01</td>
<td>51.58 ± 0.95</td>
<td>57.20 ± 0.83</td>
<td>54.06 ± 1.26</td>
</tr>
<tr>
<td>Total WBC (cells/mm(^3))</td>
<td>9830 ± 287.5</td>
<td>10015 ± 246.1</td>
<td>11767 ± 240.1</td>
<td>23683 ± 2292(^a)</td>
<td>10545 ± 211.3</td>
</tr>
<tr>
<td>Platelets (cells/mm(^3))</td>
<td>8.55 ± 0.09</td>
<td>8.58 ± 0.08</td>
<td>7.30 ± 0.50</td>
<td>7.39 ± 0.56</td>
<td>8.51 ± 0.09</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>22 ± 0.93</td>
<td>20.67 ± 0.99</td>
<td>25.67 ± 1.02</td>
<td>24.67 ± 0.43</td>
<td>22.67 ± 0.67</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>69 ± 1.01</td>
<td>70.5 ± 1.06</td>
<td>65 ± 0.86</td>
<td>68.83 ± 1.02</td>
<td>68.5 ± 0.77</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>6 ± 0.52</td>
<td>6.2 ± 0.48</td>
<td>5.2 ± 0.88</td>
<td>3.4 ± 0.56(^b)</td>
<td>6 ± 0.64</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>2.9 ± 0.17</td>
<td>2.9 ± 0.17</td>
<td>4 ± 0.26</td>
<td>3.2 ± 0.31</td>
<td>2.9 ± 0.17</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SEM (n=6), one way ANOVA followed by Tukey’s Multiple Comparison Test. 
\(^aP<0.0001, \(^bP<0.001 & \(^P<0.0001, \) Sap and Disease Vs Normal control group and ***P<0.0001, **P<0.001 Treatment Vs Disease control group on Day 11.

On Day 11, white blood cells in animals that received cyclophosphamide were significantly reduced (591.7 ± 36.46 / mm\(^3\)) relative to their baseline values (10545 ± 211.3 / mm\(^3\)). A significant increase in white blood cells was observed on final day in animals of test
group which received the *Borassus flabellifer* sap alone. Treatment with *B. flabellifer* sap and cyclophosphamide led to a significant increase (6283 ± 725.8 / mm³) in white blood cells relative to cyclophosphamide alone treated group. Interestingly, treatment with palmyra sap alone led to a significant increase from the values measured on initial day.

Fig. 3: RBC

Fig.4: Hb

Fig.5: PCV

Fig.6: MCV

Fig.7: WBC

Fig.8: Platelets
DISCUSSION
Cyclophosphamide causes numerous toxicities at different levels including myelotoxicity which cause fall in blood cells [27]. CTX administration induces oxidative stress injury, causing damage to intercellular organs, lipid peroxidation, decline in protein synthesis and mitochondrial damage [28]. Hence, there is need for affordable regimen that can protect the essential organs against the damaging effects of anticancer drugs and other chemotherapeutic agents [17].

The above nutritional benefits of *B. flabellifer* sap, the present study has revealed the myeloprotective effect on cyclophosphamide induced myelotoxicity in wistar rats. *B. flabellifer* sap showed its efficacy of having antioxidant potential and free radical scavenging activity [29]. In the present study, administration of cyclophosphamide (150 mg/kg i.p.) to wistar rats resulted in myelosuppression [15]. Major side effect of cyclophosphamide therapy is bone marrow suppression, resulting in low levels of all blood cells in hematological analysis. A significant decrease in the values of all the
hematological parameters (RBC, Hb, PCV, MCV) was observed in the cyclophosphamide treated group, compared to their baseline values measured on initial day. This is due to the effect of cyclophosphamide directly on the developing cells of reticulocytes in bone marrow. However, treatment with B. flabellifer sap increased the low levels of hematological parameters. There was no meaningful change in hematological parameters in animals of normal control group and sap control group which received sap alone except hemoglobin, which increased in sap control group significantly. Treatment with B. flabellifer sap and cyclophosphamide led to a significant increase in white blood cells relative to the cyclophosphamide alone treated group. Fascinatingly, treatment with palmyra sap alone in animals led to a significant increase in white blood cells relative to their values measured on initial day.

Hence, B. flabellifer sap offered protection to the bone marrow against the cytotoxic effects of cyclophosphamide. It could be that B. flabellifer sap exerts stimulatory effect on the hematopoietic activity of the bone marrow cells as evidenced by the significant increase in hemoglobin and white blood cells in the sap control groups when compared with the normal control group. This result may justify the consumption of the B. flabellifer sap by the natives for boosting of blood.

CONCLUSION
From the results of present study, it could be concluded that B. flabellifer sap (3.6 ml/kg) have protective effect on blood and bone marrow nucleated cells despite of high dose of cyclophosphamide. Based on present study findings, it could be proposed that B. flabellifer sap (3.6 ml/kg) together with cyclophosphamide can be use as strong candidates in preventing the cyclophosphamide-induced myelotoxicity, hemotoxicity and anemia. Hence further research is suggested to explore the exact pharmacology of the B. flabellifer sap.

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