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Haematopoietic and immunomodulatory activity of sap of *Borassus flabellifer* against cyclophosphamide mediated haematotoxicity and immunosuppression in Wistar rats

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Recent scientific evidence recommends that many of the adverse effects of chemotherapy can be prevented by remedying treatment with antioxidants, minerals, carbohydrates, proteins, and organic acids. The current research was performed to assess haematopoietic and immunomodulatory effects using sap of traditional tree, *Borassus flabellifer*, in cyclophosphamide induced haematotoxicity and immunosuppression in Wistar rats. For eleven consecutive days, the animals were orally pre-treated with *Borassus flabellifer* sap (3.6 mL/kg). These animals were intra-peritoneally injected on the sixth day with cyclophosphamide (150 mg/kg) and sacrificed five days later. On the final day of the trial, blood samples were obtained from each rat and carried out evaluation of haematological (red blood cells, haemoglobin, mean corpuscular haemoglobin concentration, reticulocytes & serum iron content) and immunomodulatory (total leucocyte count, cellular immune response & tumor necrosis factor-α) parameters. To determine their index values, each rat's thymus and spleen were separated. By elevating red blood cells, haemoglobin, mean corpuscular haemoglobin concentration, reticulocytes & serum iron content, sap of *Borassus flabellifer* demonstrated an important protective role in cyclophosphamide mediated haematotoxicity. It has also shown substantial resistance against immunosuppression caused by cyclophosphamide by increasing the immunity boosting white blood cells and decreasing the hypersensitivity reaction and tumor necrosis factor-α level at the 3.6 mL/kg dose. The current research shows that therapy with traditional *Borassus flabellifer* sap has a major protective effect against oxidative stress, haematotoxicity and immunosuppression against cyclophosphamide induced haematotoxicity and immunosuppression.

Keywords: *Borassus flabellifer* sap, Chemotherapy, Cyclophosphamide, Haematotoxicity, Immunosuppression

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There have been progresses in the treatment of multiple human diseases by chemotherapy in recent years, which has lowered death rates and improved the quality-of-life profoundly[1]. However, in addition to numerous non-malignant diseases, the efficacy of chemotherapy and other treatments such as radiotherapy, gene therapy, stem cell transplantation, etc. has been limited by their side effects and organ toxicities that have triggered more complications[2]. Chemotherapy requires the continuous use of antineoplastic agents in the treatment of cancer, which destroys uncontrolled proliferation of cancer cells, but, however, also affects normal proliferating cells which reduces their therapeutic index[3]. Widely used chemotherapeutic agents target the replication of bone marrow cells, epithelial linings and gonads (Table 1) that makes their use a matter of increasing concern[4].

In 1958, cyclophosphamide (CTX), a cyclic phosphoramide ester, was synthesized as an orally active transport form of chiormethine, the alkylating agent. It was identified on the World Health Organizations List of Essential Medicines as one of the most effective chemotherapeutic drugs[5]. The chemical is triggered by the cytochrome P-450 hepatic and its metabolites, phosphoramide mustard and acrolein are linked to its antineoplastic and other toxic adverse effects such as myelosuppression (leucopenia, neutropenia, thrombocytopenia, and anaemia), bone marrow failure and severe immunosuppression[6,7]. These adverse events contribute to severe and lethal infections, because of which over the time, its use has declined. Myelosuppression is also categorized by diminished immunity and haematopoietic function in patients

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Table 1 — Adverse effect of various chemotherapeutic agents

Drug	Class	Uses	Adverse Effects
Doxorubicin	Anthracycline	Solid tumors haematologic malignancies	Acute and chronic cardiotoxicity; cardiomyocyte loss replaced by fibrotic tissue leads to heart failure
Daunorubicin	Anthracycline	Acute leukemia	Cardiomyopathy
Epirubicin	Anthracycline	Breast	Cardiomyopathy
Cyclophosphamide	Alkylating agent	Breast, lung, myeloma, lymphoma, leukemia	Anemia due to bone marrow cell toxicity; hemorrhagic cystitis
Cisplatin	Alkylating agent, metal salt	Head, neck, bladder, cervical	Renal and neurotoxicity, myelosuppression, fatigue.
Oxaliplatin	Alkylating agent, metal salt	Gastric, colorectal	Myelosuppression, N/V/stomatitis, neurotoxicity; peripheral neuropathy
Carboplatin	Alkylating agent, metal salt	Ovarian, smallcell lung	Nausea/vomiting, myelosuppression
Methotrexate	Antimetabolite antifolate	Head, neck, breast, leukemia, lymphoma	Hepatotoxicity, low WBC
Fluorouracil	Antimetabolite antifolate	Anal, breast, esophageal, colorectal	Diarrhea, neutropenia, myelosuppression, fatigue
Vinblastine	Mitotic inhibitor	Hodgkin's, lung, bladder, testicular	Leucocyte and platelet damage, GI problems, hypertension, muscle cramps, vertigo
Vincristine	Mitotic inhibitor	Acute leukemia, Hodgkin's	Neurotoxicity (peripheral neuropathy), constipation
Docetaxel	Mitotic inhibitor	Breast, lung, head, neck, prostate, stomach	Myelosuppression, fatigue, nausea, vomiting, palmar/plantar syndrome, neuropathy

receiving chemotherapy with CTX or another chemotherapy agent. Hematologic toxicities caused by these chemotherapeutic therapies are now the underlying causes for mortality and morbidity in the management of malignancy[8,9].

Chemotherapy toxicities are conventionally treated by minimizing the dosage of chemotherapeutic drugs, which unfortunately decreases the adequacy of treatment, the use of the reinforcing factor of granulocyte colony and oligonucleotides[10-12]. However, the latter steps are expensive and achieve undesirable and unsatisfactory outcomes. A few modern approaches are being developed for the management and treatment of toxicity caused by CTX and chemotherapy. Latest clinical evidence shows that many of the adverse effects of cancer can be treated by treating with vitamins, nutrients, fats, proteins, and organic acids (Table 2). These nutrients have useful antioxidant functions that can neutralize hazardous free radicals such as O_2, H_2O_2 and OH generated by chemotherapeutic agent metabolism[12-23]. Considering the drastic rise in the number of malignant patients, there is a necessity for reasonable and conveniently available antioxidants that can secure the vital organs from toxicities of chemotherapeutic drugs.

Traditionally known as Palmyra palm, *Borassus flabellifer* L (Arecaceae) is a native robust tree found in the tropical Africa region that has been grown, cultivated, and naturalized throughout India. Modern research has proven that sap, flowers, fruits, leaves and seeds of palmyra palm have benefits that resist diseases. A cooling sugary sap called Neera, drops out from the young immature male or female inflorescence of the tree, and is collected before morning or sunrise. It is a nutritious health drink with starch, fats, lipids, amino acids, steroids and 0% alcohol. It is an opulent source of sugars, nutrients, vitamins, and organic acids. It has a sweet sugar taste and a feeling that is extremely cool and has a significant role in Ayurvedic medicine. It has distinct physicochemical features that have much health benefits[24,25]. Sap is employed as a tonic, laxative, diuretic, and stimulant. It also has the antiphlegmatic, anti-inflammatory, antibacterial, analgesic, cytotoxic, antipyretic, hypoglycemic, antioxidant, antihelmintic, antifungal, and amoebicide properties[26]. It is said that sugar made from this sap can counteract poisoning. For the prevention of typhoid & chickenpox, fresh sap is used by local people in Tamil Nadu & Kerala states of India[27].

Therefore, based on the antioxidant and nutritional advantages of *Borassus flabellifer* sap, the current research was conducted to assess the haematopoietic and immunomodulatory role of *Borassus flabellifer* fresh sap in the haematotoxicity and immunosuppression caused by cyclophosphamide in wistar rats.

Material and Methods

Chemicals and reagents

Cyclophosphamide (Phosmid) was acquired from NEON Laboratory Ltd., Mumbai, Gower's solution &

Table 2 — Various remedying therapies for treating anticancer toxicities

Treatment	Class	Chemotherapeutic Regimen	Outcomes	Reference
1,8-cineole, exopolysaccharide and ellagic acid	Terpene	Cyclophosphamide	Pretreatment fundamentally diminished serum markers of liver and heart, prevents ROS generation and reduced TGF-β1 in liver and heart tissues.	Abdallah *et. al*., 2019[14]
Iron	Mineral	Various Chemotherapeutic agents	Recommended the utilization of IV iron alongside ESA in patients with chemotherapy or immunotherapy.	Rodgers *et. al*., 2019[15]
Sugars and vitamins	Antioxidant	Cyclophosphamide	Pretreated in mice have altogether secured cyclophosphamide induced nephrotoxicity.	Eltantawy *et. al*., 2018[16]
Magnesium	Mineral	Cisplatin	IV regimen prevents cisplatin brought nephrotoxicity in patients with cancer.	Kimura *et. al*., 2018[17]
Lavandula officinialis	Antioxidant and mast cell degranulation inhibitory properties	Cyclophosphamide	Pretreated rats have shown significant nephroprotective effect which was further established by the histological examination of the kidneys.	Sadeghi *et. al*., 2018[18]
Hypericum triquetrifolium	Flavonoids and antioxidants	Cyclophosphamide	Methanolic extracts significantly reduced hepatotoxicity, myelotoxicity and haematotoxicity in rats.	Yildiz *et. al*., 2018[19]
Glutamine	Amino Acid	5-Fluorouracil, Paclitaxel	Supplementation significantly defends against mucositis, myalgias& neurotoxicity	Pandey *et. al*., 2012[20]
Tocopherol	Vitamin E (Antioxidant)	Paclitaxel	Tremendous protection in patients with cancer and nerve damage.	Argyriou *et. al*., 2006[21]
Cyanocobalamin & Folate	Vitamin B 12 & Vitamin B 9	Doxorubicin / cyclophosphamide	Decline in neutrophil brought about by chemotherapy was improved by dietary supplementation with multivitamins.	Branda *et al*., 2004[22]
Ascorbic Acid	Vitamin C (Antioxidant)	Cisplatin	Significant protective role against organs toxicities was reported without obstructing the cancer treatment.	Goel *et. al*., 1999[23]

WBC diluting fluid from S.D. Fine Chemicals Ltd., Drabkin solution from ARKRAY Healthcare Pvt. Ltd., Mumbai and Leishman's stain procured from Nice Chemicals Pvt. Ltd., Kochi, India. Other chemicals used were of analytical grade from normal sources and were collected. Serum iron content was measured using Coral Clinical Systems Biochemical Iron kitand TNF-α from ARKRAY Healthcare Pvt. Ltd., Mumbai, India.

Collection and physical assessment of sap

The fresh sap was collected from the Jalpally village of Ranga Reddy District, Hyderabad, India, depending on the geographical location of the *Borassus flabellifer* tree (Fig. 1). It was collected early in the morning before sunrise and processed in a cold state at temperatures below 0°C to prevent the sap from destroying the chemical constituents. The sap was transferred to the testing laboratory and its physical properties were tested for purity. As per the literature description, the colour, odour, taste and pH were assessed for differentiating sap from fermentation (Table 3)[28].

Fig. 1 — Collection of *Borassus fabellifer* sap

Experimental animals

For the research, healthy adult wistar rats of either sex weighing 200-250 g were used. The rats were kept in cages of polypropylene with a sterile paddy husk as bedding. They were acclimatized with free access to normal pellets such as basal diet and water *ad libitum* to the laboratory conditions of ambient

Table 3 — Physical properties of fresh and fermented *Borassus flabellifer* sap

Properties	Colour	Odour	Taste	pH
Fresh Sap	Oyster White	Pleasant	Sweet	7.2
Fermented Sap	Turbid	Alcoholic	Burning & Unpleasant	5.9

temperature (25±2°C), 30-60% humidity and 12 h light-dark cycles. All experiments were performed as per the guidelines of the Institutional Animal Ethics Committee, G. Pulla Reddy College of Pharmacy (GPRCP/IAEC/23/19/02/PCL/AE-2-Rats-M/F-24), Hyderabad, India; complying with the guidelines as established by the committee for the purpose of Control and Supervision of Experiments in Animals (CPCSEA), Government of India, regarding the care and use of laboratory animals in scientific experiments in the month of Febraury, 2019.

Cyclophosphamide induced haematotoxic and immunosuppression dose

Cyclophosphamide dose of 150 mg/kg b.w. for inducing haematotoxicity and immunosuppression was reliable with the literature evidence, suggesting a single dose administration of 100 mg/kg b.w.**,** 150 mg/kg b.w.**,** or 200 mg/kg b.w.[19,29]. Hence, haematotoxicity and immunosuppression was induced in animals by administrating cyclophosphamide with a single dose of 150 mg/kg intraperitoneally (i.p.) on 6th day of experiment, accompanied by administration of test substance for remaining experimental days.

Selection of *Borassus flabellifer* sap dose

Based on previous literature evidence from direct human trials, the dosage of sap of *Borassus flabellifer* was selected[30,31]. The animal dosage has been extrapolated from human dose[32].

Experimental design

The experimental animals (n=24) were randomly divided into 4 groups containing six animals each. The animals were divided into the groups as follows: Group I (Normal Control) animals received normal saline (0.9%), 1 mL/kg, orally (p.o.). Group II (Disease Control) animals received CTX, 150 mg/kg, intraperitoneally (i.p.), on the 6th day. Group III (Sap Control) animals received sap of *Borassus flabellifer*, 3.6 mL/kg b. w., p.o. Group IV (Treatment): 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 the final day of experiment, the blood was collected from each rat by retro-orbital plexus and haematological and biochemical parameters were estimated.

Estimation of haematological parameters

Blood samples from experimental animals were collected into EDTA (ethylenediamine-tetraacetate) bottles and analyzed using standard procedures. Red blood cell (RBC) count was performed according to the procedure developed by Cheesbrough & McArthur, 1976[33]. Hemoglobin (Hb) was measured by spectrophotometric method described by Drabkin & Harold, 1932[34]. Percentage of reticulocytes was carried out by cresyl blue stain and the concentration of reticulocyte/µL was determined based on whole red blood cells[35]. Serum iron content was measured spectrometrically by using Ferrozine/$MgCO_3$ method[36]. Mean corpuscular haemoglobin concentration (MCHC: ratio of weight of hemoglobin to the blood volume) was calculated from the following formula[37].

$$\text{MCHC (\%)} = \frac{\text{Hemoglobin}}{\text{Haematocrit}} \times 100$$

Estimation of immunomodulatory parameters

Total leucocyte count (TLC) was measured according to Cheesbrough & McArthur, 1976 method[33] and differential leucocyte count (DLC) was performed according to Blumenreich, 1990[38]. The amount of tumor necrosis factor - alpha (TNF-α) was determined with the method described by Barone, *et al*., 1997[39].

Cellular immune response was assessed by footpad reaction test. After measuring volume of footpad of both legs on last day of experiment, sheep red blood cells (SRBC: $0.025x10^9$ cells) were injected in left paw and 0.025 mL of saline was injected in right paw. After 24 h, the paw volume was again measured for increase or decrease in the volume. The increase in paw volume was considered as an index of cell-mediated immunity (delayed type hypersensitivity)[40].

Thymus and spleen index

After completion of the study, thymus and spleen of each rat were isolated, cleaned with normal saline, weighed and spleen and thymus index were determined for each animal.

Statistical analysis

Data obtained from various parameters were statistically analyzed to determine significant differences in data of various groups with one way analysis of variance (ANOVA) followed by Tukey's multiple comparison test in Graphpad Prism 7.0 software; p-values less than 0.5 were considered significant. The values are expressed as mean±SEM.

Results

Physical assessment of *Borassus flabellifer* sap

To avoid microbial deterioration, the palmyra sap obtained in the morning was transferred under cooling conditions to the testing laboratory. To distinguish from the fermented phase, the physico-chemical properties of fresh sap were measured (Table 4).

Effect of sap of *Borassus flabellifer* on haematological parameters

The blood of experimental animals was estimated for various haematological parameters i.e., RBC, Hb, MCHC, reticulocytes & serum iron content and the effect of sap of *Borassus flabellifer* on these parameters has been shown in Table 5. In cyclophosphamide treated animals, a significant ($p<0.001$) reduction in red blood cells, haemoglobin, MCHC, reticulocytes and serum iron content were observed relative to the normal control group. This was suggestive of haematotoxic anemia in cyclophosphamide treated animals. However, these variations in blood parameters in animals treated with *Borassus flabellifer* sap have significantly ($p<0.001$) returned to normal.

Table 4 — Evaluation of *Borassus flabellifer* sap

S. No.	Properties	Fresh Sap
1	Color	Oyster White
2	Odour	Agreeable
3	Taste	Sweet
4	pH	6.9

Also, significant rise in the haemoglobin ($p<0.01$) and serum iron content ($p<0.001$) in sap alone treated group was found as compared to the standard control group. Due to the treatment with *Borassus flabellifer* sap, the animals in the treatment group exhibited pronounced protective effects on cyclophosphamide mediated haematotoxicity.

Effect of sap of *Borassus flabellifer* on total and differential leucocyte counts

The effect of sap of *Borassus flabellifer* on total and differential leucocyte countshas been shown in Table 6. In cyclophosphamide treated animals, a significant ($p<0.001$) reduction in the overall count of leucocytes with specifically monocytes & neutrophils were observed relative to the normal control group. Treatment with *Borassus flabellifer* sap, however, contributed to a significant ($p<0.001$) rise in the number of leucocytes, lymphocytes, and neutrophils relative to the animals treated with cyclophosphamide alone. A highly significant increase in the number of leucocytes ($p<0.001$) was also observed on the last day of experiment in sap-treated animals.

There was no noticeable improvement in eosinophils and basophils except for lymphocytes ($p<0.001$), monocytes ($p<0.01$) and neutrophils ($p<0.001$), which was observed to be increased on the final day in animals treated with sap alone, compared to the normal group.

Table 5 — Effect of *Borassus flabellifer* sap on haematological parameters

Groups	Parameters				
	RBC (million/mm^3)	Hb (mg/dL)	MCHC (%)	Reticulocyte (%)	Serum Iron (µg/dL)
Normal (Saline)	8.45±0.22	13.13±0.12	31.58±1.54	5.16±0.23	79.81±1.25
Disease (CTX)	5.57±0.17$^{\alpha}$	7.14±0.12$^{\alpha}$	25.75±1.68$^{\alpha}$	0.81±0.14$^{\beta}$	62.14±1.64$^{\alpha}$
Sap (*Borassus flabellifer* Sap)	9.85±0.27	17.02±0.19^{b}	34.74±2.05	5.24±0.21	86.11±1.57^{a}
Treatment (*Borassus flabellifer* Sap + CTX)	8.15±0.15	13.74±0.14***	34.98±1.75***	4.95±0.19***	81.05±1.48***

RBC: red blood cells, Hb: Hemoglobin, MCHC: mean corpuscular hemoglobin concentration.

Values are expressed as mean±SEM (n=6), $^{a}p<0.001$, $^{b}p<0.01$ & $^{\alpha}p<0.001$, $^{\beta}p<0.01$ compared to the normal control group and ***$p<0.001$ compared to the disease control group using one way analysis of variance (ANOVA) followed by Tukey's multiple comparison test.

Table 6 — Effect oSf *Borassus flabellifer* sap on immunomodulatory parameters

Groups	Parameters					
	Total Leucocytes (cells/mm^3)	Lymphocyte (%)	Neutrophil (%)	Monocyte (%)	Eosinophil (%)	Basophil (%)
Normal (Saline)	11024±235.2	71.5±1.06	20.67±0.99	6.1±0.48	2.9±0.17	1±0
Disease (CTX)	745.2±853.4$^{\alpha}$	70±0.73	24.5±0.77$^{\alpha}$	1±0$^{\alpha}$	1±0	0±0
Sap (*Borassus flabellifer* Sap)	24754±54.7^{a}	67.83±1.02^{a}	24.67±0.43^{a}	3.4±0.56^{b}	3.2±0.31	2±0
Treatment (*Borassus flabellifer* Sap + CTX)	12845±652.3***	75±0.97***	19±0.87***	3.5±0.23*	1.7±0.22	1±0

Values are expressed as mean±SEM (n=6), $^{a}p<0.001$, $^{b}p<0.01$ & $^{\alpha}p<0.001$ compared to the normal control group and ***$p<0.001$, *$p<0.05$ compared to the disease control group using one way analysis of variance (ANOVA) followed by Tukey's multiple comparison test.

Effect of sap of *Borassus flabellifer* on cellular immune response

As seen in Fig. 2, treatment with *Borassus flabellifer* sap showed an important beneficial function in cell-mediated immunity to cyclophosphamide. In addition to inflammation, a significant ($p<0.001$) rise in the volume of paw of animals was found in the cyclophosphamide-treated animals. In contrast to the disease control group, a significant ($p<0.001$) drop in paw volume was found in the animals in the treatment group. The result observed in the test group was very significant when compared to the normal and disease control group.

Effect of sap of *Borassus flabellifer* on cytokine level (TNF-α)

Fig. 3 indicates the cytokine level of TNF-α in the present study. Compared to the normal control group, a significant ($p<0.001$) rise in TNF-α was found in cyclophosphamide treated animals. However, treatment with *Borassus flabellifer* sap led to significant ($p<0.001$) decrease in the TNF-α levels in animals as compared to cyclophosphamide alone treated animals. Further, a significant ($p<0.001$) decrease in TNF-α level was found in the sap-alone treated animals.

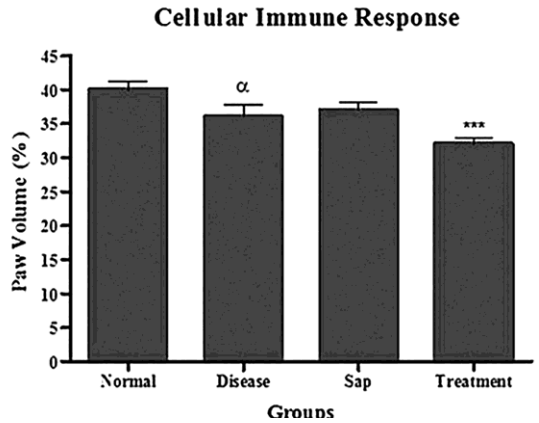

Fig. 2 — Effect of *Borassus flabellifer* sap on cellular immune response Values are expressed as mean±SEM (n=6), $^{\alpha}p<0.001$ compared to the normal control group and ***$p<0.001$ compared to the disease control group using one way analysis of variance (ANOVA) followed by Tukey's multiple comparison test.

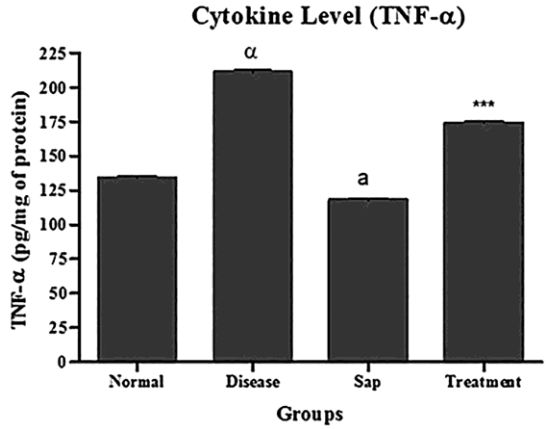

Fig. 3 — Effect of *Borassus flabellifer* sap on cytokine level (TNF-α) Values are expressed as mean±SEM (n=6), $^{\alpha}p<0.001$ & $^{a}p<0.001$ compared to the normal control group and ***$p<0.001$ compared to the disease control group using one way analysis of variance (ANOVA) followed by Tukey's multiple comparison test.

Discussion

In certain kinds of advanced cancer and disease complications, chemotherapy is curative, but various toxicities are often triggered by chemotherapeutic agents. In the management and treatment of different malignancies and other disorders, chemotherapeutic drugs are used either alone or in conjunction with other drugs or treatments. They target bone marrow regions and further raise the risk of developing serious and prolonged cytopenias and immunosuppression marked by a substantial decline in blood cells (including WBC)[41]. Chemotherapeutic agents promote generation of free radicals that react with biomolecules to yield different types of secondary radicals that cause chain reactions together with oxygen. Cellular membranes are vulnerable to oxidation by free radicals owing to the presence of high concentrations of fatty acids in lipid materials. Lipid peroxidation is caused by the association of free radicals and membrane lipids, resulting in cross-linkage of membrane proteins and the development of lipid-protein, a lipid-DNA adduct that is known to be detrimental to cell structure, leading to toxicity[35,42].

Cyclophosphamide is a chemotherapeutic drug used for the prevention of infectious disorders and numerous malignancies, including ovarian and breast cancer, Burkitt's lymphoma, myeloid leukaemia, retinoblastoma, lung cancer, sarcoma, neuroblastoma, retinoblastoma, and multiple myeloma. The undesired conditions that occur during CTX chemotherapy are haematotoxicity and immunosuppression. The inhibition of DNA replication that suppresses bone marrow cells to generate peripheral blood cells, including reticulocytes, is one of the reported mechanisms of haematological toxicity induced by CTX. It is also important to note that the production and maturation of peripheral blood cells depends on the activity of the bone marrow haematopoietic stem cells and progenitor haematopoietic cells. Furthermore, it is well known that the circulating peripheral blood cells have a defined and restricted

life span and therefore their continuous replacement is necessary for the body system's successful homeostasis[43]. Therefore, an inexpensive regimen is needed that can protect the vital organs from the adverse effects of anticancer drugs and other chemotherapeutic agents.

Antioxidants play a significant function and can protect cells from free radicals and chemotherapy-induced toxicities. They bind to free radicals and prevent substrates involved in the most critical cellular reactions from being degraded. Antioxidant therapies can thus reduce the destructive effects of chemotherapy or radiotherapy by reducing oxidative damage. By interacting with oxidising free radicals, they can prevent cellular damage to normal organs and tissues[13].

The fresh sap of *Borassus flabellifer* is a rich source of carbohydrates, minerals (magnesium, sodium, calcium, potassium, copper and zinc), vitamins (retinol, niacinandriboflavin) and organic acids with proven antioxidant agents. Fresh sap has antioxidant potential and free radical scavenging activity that has already been reported[44]. Succinic acid in the sap has been found in highly significant amounts. In sap, which has already been shown to possess antioxidant activities, numerous other organic acids such as citric acid, tartaric acid, malic acid, lactic acid, fumalic acid and pyrogalic acid were also documented. It contains large quantities of iron, phosphorus, and ascorbic acid that are helpful in the haematopoiesis process. Fresh sap is more beneficial than any of the fruit juices that are commercially sold in India. It has a glycemic index of 35, meaning diabetic patients can use it[39].

The haematological and immunomodulatory activity of *Borassus flabellifer* sap against haematotoxic and immunosuppressive wistar rats caused by cyclophosphamide has been demonstrated in the current research. During and after research completion, no morbidity of animals was detected in the animal group administered with only *Borassus flabellifer* sap. Hence, it was found safe to be used at the measured dosage in the experimental animals.

Bone marrow is one of the essential components of the body system, responsible for the development of RBC, Hb, leucocytes and thrombocytes for sustaining immunity and other homeostatic functions. In the current research, cyclophosphamide administration (150 mg/kg, i.p.) caused pronounced haematotoxicity and immunosuppression in wistar rats due to the death of immature bone marrow cells caused by the activity of CTX toxic metabolites. However, a substantial decrease in RBC, Hb, MCHC, reticulocytes and serum iron content was found in the community treated with CTX alone, which was prevented by *Borassus flabellifer* sap pre-treatment.These outcomesestablished indirect evidence for the modulation and increment of haematological parameters by administration of *Borassus flabellifer* (3.6 mL/kg) sap which has antioxidant potency.

Undeveloped RBCs, referred to as reticulocytes, are released from marrow cells and circulate in the blood for at least two days prior to RBC maturation. The quantity of reticulocytes mimics the quantity of iron required for haemoglobin development, for which it is considered an important haematological marker[45]. In their progression period, reactive oxygen species (ROS) generation and oxidative injury are responsible for damage to reticulocytes. This contributes to poor counts of RBC and other blood cells in CTX treated group. However, the outcomes of the present study indicated that *Borassus flabellifer* sap (3.6 mL/kg) substantially increased the amount of RBC, mainly 5 days after administration of CTX. *Borassus flabellifer*'s fresh sap may then activate the mechanism of haematopoiesis in bone marrow.

The cells involved in the body's immune protection consist mostly of lymphocytes, monocytes, and neutrophils. In the current research, administration of CTX increased ROS and contributes to a decline in immune cell levels in CTX treated animals. This was perhaps due to the degradation by the crosslinking process of CTX of the developing lymphocytes and other immune cells. *Borassus flabellifer* sap (3.6 mL/kg) treatment prevented the decline and elevated the lymphocytes, monocytes and neutrophils significantly indicating potent immunomostimulatory effect.

The paw edema test was used to assess cellular immune response by delayed type hypersensitivity. Delayed type hypersensitivity is a response to type IV hypersensitivity that occurs when sensitized T cells are triggered by any antigen[46]. The findings of the current research revealed that the hypersensitivity reaction was greatly decreased by treatment with *Borassus flabellifer* sap (3.6 mL/kg), as shown by a decrease in paw volume.

TNF-α is primarily released by macrophages and is involved in the acute inflammation process by neutrophil recruitment and arachidonic acid

metabolism activation[47]. The findings of this study showed that *Borassus flabellifer* sap (3.6 mL/kg) greatly decreased the release of TNF-α pro-inflammatory cytokine in macrophages.

The overall findings of the present study revealed that the haematological and immune response was significantly improved when *Borassus flabellifer* sap (3.6 mL/kg) was administered as demonstrated by the ability of different markers to activate the immune response. As previously mentioned, the organic acids present in the fresh *Borassus flabellifer* sap acts as antioxidants may be responsible for the protective effects that have prevented the degradation of immature reticulocytes and immune cells by cyclophosphamide metabolites. Also, the carbohydrates and minerals present in the sap may be responsible for increasing the blood cells and immunity. The oral administration of *Borassus flabellifer* sap therefore indicated possible protective effects against toxic changes in haematological and immunomodulatory parameters caused by free radicals of CTX metabolites.

Conclusion

To protect normal cells and tissues from chemotherapy-treated oxidative damage, presently cyclophosphamide, the combination of chemotherapy regimens with antioxidative and cytoprotective agents can be useful. It can be inferred from the findings of the current research that *Borassus flabellifer* sap (3.6 mL/kg) defends the bone marrow tissue with its antioxidant and free radical scavenging action as it contains high amounts of organic acids against oxidative damage induced by a high dose of cyclophosphamide. Further, the minerals of sap enhance haematopoiesis and boost immunity. Based on present study findings, it could be proposed that, along with CTX, *Borassus flabellifer* sap can be used as a good candidate to avoid CTX-induced toxicities as the sap is easily available and accessible.

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Conflict of Interest

The authors declared no conflicts of interest.

Author's Contributions

MAS and RP conceived of the presented idea and designed the study. RP helped supervise the project. MAS carried out the experiments. MAS wrote the original draft of the manuscript with support from RP. RP reviewed and edited the manuscript. All authors discussed the results and contributed to the final version of the manuscript.

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