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Haemopoietic Potential of Tannin Fractionates of *Vitex doniana* Leaf on Nitrosobis (2-Oxopropyl) Amine Comobidity in Docetaxel-Induced Myelosuppression in Wistar Rat

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Authors' contributions

This work was carried out in collaboration between both authors. Author GEA wrote the protocol, supervised the research and drafts of the manuscript and managed the analyses of the study. Author IEA designed the study, managed the literature search, performed the statistical analysis and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: This study investigated the haemopoietic potential of tannin fractionate of Vitex doniana leaf against nitosobis (2-oxopropyl) amine comorbidity in docetaxel-induced bone marrow suppression.
Study Design: This is an experimental research.
Place of Research: University of Nigeria, Enugu campus.
Methodology: The male Wistar rats used in this experiment were twenty-eight in number, and they were grouped into 7, with each group having four rats. Group 1 served as control, and received 1ml of normal saline, while groups 2-7 were treated with Nitrosobis (2-oxopropyl) amine 5 mg/kg daily for 2 weeks. Then groups 3-7 were treated with 8 mg/Kg of docetaxel weekly for 2 weeks. And groups 4, 5 and 6 also received 250 mg/Kg and 500 mg/Kg and 1000 mg/kg of tannin, respectively, daily for 2 weeks. Group 7 received 40 mg/Kg of fesolate daily for 2 weeks.

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Results: The haemoglobin concentration and white blood cell count of rats in the groups treated with Nitrosobis (2-oxopropyl) amine alone (group 2) and Nitrosobis (2-oxopropyl) amine plus Docetaxel (group 3) showed statistically significant reduction (p=.05) in number when compared with the group treated with normal saline (group 1). The haemoglobin concentration and white blood cell count of the rats in the groups treated with 250 mg/kg, 500 mg/kg, and 1000 mg/kg of the tannin fractionate, in addition to the Nitrosobis (2-oxopropyl) amine and Docetxel (i.e. groups 4, 5, and 6, respectively) showed statistically significant dose dependent increase in number, with the group treated with 1000mg/kg showing the highest increment (p=.05). The cells in the bone marrow show significant reduction in number in the rats treated with Nitrosobis (2-oxopropyl) amine (group 2) and Nitrosobis (2-oxopropyl) amine plus docetaxel (group 3) when compared with the rats in group 1 (treated with normal saline). With the addition of graded doses of the tannin fractionate, 250 mg/kg, 500 mg/kg, and 1000mg/kg (i.e. groups 4, 5, and 6, respectively), the number of cells in the bone marrow showed statistically significant increase when compared to group 3 (treated with Nitrosobis (2-oxopropyl) amine plus docetxel), with the rats in the group treated with 1000 mg/kg of tannin fractionate (group 6) having the highest increment (p=.05).
Conclusion: Tannin obtained from *Vitex doniana* leaf extract increases the haemoglobin concentration in dose dependent manner. It also increases the white blood cell count, and number of proliferating bone marrow cells, following suppression by Nitrosobis (2-oxopropyl) amine and Docetaxel. So, this tannin obtained from *Vitex doniana* leaf extract may be useful in clinical practice to cushion the myelosupression, anaemia and leukopenia that are associated with use of docetaxel in treatment of malignancies.

Keywords: Tannin; Docetaxel; leukopenia; Vitex doniana; myelosupression; anaemia; bone marrow.

1. INTRODUCTION

Bone marrow suppression is a common side effect of use of chemotherapeutic agents [1]. This suppression of the bone marrow leads to reduction in the production of precursors cells that are responsible for the production of white blood cells, red blood cells (which contains haemoglobin), and other types of blood cells, which will ultimately lead to low peripheral levels of white blood cell count (leukopenia), low red blood cell count (anaemia) and other haematological derangements. The patient with these derangements are predisposed to infection, fatigue and clotting abnormalities (easy bleeding) [2]. These life-threatening events are to be considered when any chemotherapeutic agent is to be used, as the patient may die of complications of the treatment with the chemotherapeutic agent instead of the disease [1]. Nitrosobis (2-oxopropyl) amine is one of the agents that can be used for the induction of early prostate cancer in experimental rats [3]. Docetaxel is used singly or in combination with other agents in treatment of malignancies, such as prostate cancer [4]. It can also be used in treatment of oesophageal tumour, and breast cancer [5,6]. Apart from nausea and vomiting, and neutropenia, docetaxel can also cause other side effects, which include hypersensitivity reactions, oedema and abnormalities in the nail [7]. Docetaxel should not be given to patients with suboptimal serum levels of white blood cell, platelet, and haemoglobin concentration [8]. When a patient has suboptimal serum levels of these blood parameters, there exists available options available for optimization prior to administration of docetaxel, and these options include use of fesolate, Vitamin C and E, blood transfusion (in extreme cases) [9]. Some blood components, such as granulocyte colony stimulating factor, are not readily available in resource poor countries and centres, and when available may not be able to get the blood to optimal level, and thus, treatment is hampered [10]. Several elements that are beneficial to man, such as tannins, phosphorus, phytates, potassium, are found in large quantities in leaves [11]. Some of these leaves are being used by rural dwellers and in traditional medicine for treatment of anaemia with huge success [12,13]. Vitex doniana is one of these leaves, and studies have attributed its haemopoetic effect to one of its contents, tannin [14,15]. Literature search did not show that there is any study on the effect of tannin fractionate from Vitex doniana leaf on the bone marrow cells and haematological parameters of Wistar rats treated with docetaxel. The aim of this study was to investigate the haemopoietic potential of tannin fractionates of V*itex doniana* leaf on nitrosobis (2-oxopropyl) amine comorbidity in Docetaxel-induced myelosuppression in Wistar rat.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

Fresh leaves of *Vitex doniana* were obtained from *Vitex doniana* tree in University of Nigeria, Enugu campus. The leaf was authenticated by the department of botany, University of Nigeria, Nsukka.

2.2 Preparation of Extract

The stalks of the leaves were removed, then the leaves washed with distilled water and subsequently air-dried under shade at room temperature. Then pulverized to fine powdered form using mortar and pestle. The powdered material was then sieved using sieve with little pores to remove the ungrounded fibres [16]. One thousand grams of the powdered leaves was extracted exhaustively, each time. The aqueous extract was filtered using Whatman No. 2 filter paper. And then concentrated with a rotary evaporator at 40°C and stored in refrigerator at 4°C until isolation of tannin [17].

2.3 Isolation of Tannin

The mobile phase was prepared by mixing methanol and water in ratio of 50:50 and filtered through 0.2µm filter, using vacuum pump and sonicated for 30 min. Preparation of calibration curve of tannic acid was by dissolving Tannic acid (10 mg) in 10 ml of mobile phase to prepare stock solution with concentration of 1000 microgram/ml. A series of dilutions with concentration of 20, 30, 40, and 50 µg/ml was prepared by taking aliquots of 0.2, 0.3, 0.4, and 0.5 ml of stock solution (1000 microgram/ml) and diluted up to 10 ml with mobile phase. Each dilution (20 microlitre) was injected with the help of a syringe in triplicate and the area under curve at 270 nm was recorded. 10 mg of sample was dissolved in 10 ml of mobile phase and allowed to stand for 8 hours with occasional stirring and filtered through 0.2µm filter and sonicated for 30 min. Quantification was carried out using an absolute calibration curve method with standard solutions of Tannic acid [18].

2.4 Procurement of Rats

Twenty-eight male Wistar rats with average weight of 180g were procured from animal house of Department of Anatomy, University of Nigeria, Enugu campus. The rats were handled carefully according to the protocol of the Committee for the purpose of control and supervision of experiments on Animals. They were housed in netted iron cages and kept under standard laboratory conditions, where temperature was 25°C, humidity 60-70% and they had 12-hour light and dark cycles throughout the experiment. The rats were grouped into 7. Each group had 4 rats and was placed in separate clean cages in the animal house of Department of Anatomy, University of Nigeria, Enugu Campus. They had two (2) weeks to acclimatize. During this time, they had free access to rat chow and water.

2.5 Administration of Agents

Group 1 served as normal control and was given 1ml Normal saline, orally, throughout the experiment; Group 2-7 were given Nitrosobis (2-oxopropyl) amine 5mg/kg daily for two weeks. Then Groups 3-7 received Docetaxel 8 mg/kg weekly for two weeks. Groups 4, 5 and 6 further received Tannin fractionates at 250 mg/kg, 500 mg/kg and 1000 mg/kg, respectively, while group 7 received fesolate at 40 mg/kg, at same time as docetaxel, as shown in Table 1.

2.6 Sample Collection

At the end of the experiment (day 28), the rats were anaesthetized using intraperitoneal thiopentone at 50mg/kg. Blood samples were then collected from the retro-orbital vein of the rats for haematological analysis [19]. The rats were then sacrificed and the bone marrow aspirated and slide smear made immediately and fixed before histological analysis using Giemsa stain.

Table 1. Administration of agents

Groups	1-2 weeks	2-4 weeks
1	Normal saline 1 ml	Normal saline 1ml
2	Nitrosobis amine 5 mg/kg	
3	Nitrosobis amine 5 mg/kg	Docetaxel 8 mg/kg
4	Nitrosobis amine 5 mg/kg	Docetaxel 8 mg/kg + Tannin 250 mg/kg
5	Nitrosobis amine 5 mg/kg	Docetaxel 8 mg/kg + Tannin 500 mg/kg
6.	Nitrosobis amine 5 mg/kg	Docetaxel 8 mg/kg + Tannin 1000 mg/kg
7	Nitrosobis amine 5 mg/kg	Docetaxel 8 mg/kg + Fesolate 40 mg/kg

2.7 Sample Analysis

The haematological parameters were obtained for each blood sample using ERMA, PCE-210, automated haematology Analyzer (made in Japan). The histological slides of the bone marrow specimens were prepared using the standard histological techniques with Giemsa stain. The slides were analyzed using a light microscope, and computer assisted stereology was used for quantitative analysis of the proliferating cells.

2.8 Statistical Analysis

This was done using Statistical Package for Social Sciences (SPSS) version 25. p-value of .05 or less was considered significant.

3. RESULTS

3.1 Results of Haemoglobin Concentration and White Blood Cell Count

There was statistically significant decrease in the haemoglobin concentration, and serum level of white blood cells in the group treated with Nitrosobis (2-oxopropyl) amine plus docetaxel (group 3), when compared to the normal control, as shown in Table 2. The haemoglobin concentration and white blood cell count were observed to have increased significantly following addition of graded doses of tannin fractionate of *Vitex doniana* at doses of 250 mg/kg (group 4), 500 mg/kg (group 5), and 1000 mg/kg (group 6), with the group treated with 1000 mg/kg of tannin fractionate of Vitex doniana (group 6) observed to have the highest increase (p=.04) in haemoglobin concentration and white blood cell count, when compared with group 3 (treated Nitrosobis (2-oxopropyl) amine plus docetaxel), as shown in Table 2.

The group treated with fesolate 40mg/kg (group 7) were observed to have a significant increase in haemoglobin concentration and white blood cells count, when compared to the groups treated with Nitrosobis (2-oxopropyl) amine plus docetaxel (group 3). However, this increment in haemoglobin concentration and white blood cell count was not up to that observed in the group treated with 1000 mg/kg of Tannin fractionate of Vitex doniana (group 6), as this group 6 showed statistically significant increase (p=.05) in haemoglobin concentration and white blood cell count when compared to the group 7 (treated with fesolate 40 mg/kg). This is shown in Table 2.

3.2 Histological Analysis

The histology of the bone marrow, following quantification using computer assisted stereology, showed significant decrease in number of proliferating cells in the group treated with Nitrosobis (2-oxopropyl) amine plus docetaxel (group 3), when compared to normal control (group 1), as shown in Fig. 1a. There was progressive increase in the number of proliferating cells stained with Giemsa stain in the bone marrow in the groups treated with 250 mg/kg, 500 mg/kg, and 1000 mg/kg of tannin fractionate of Vitex doniana leaf (groups 4, 5, and 6), with statistically significant increase (p=.05) observed in group 6 (treated with 1000 mg/kg) when compared with group 3 (Nitrosobis amine plus docetaxel). This is as shown in Fig. 1d, 1e, and 1f. The group treated with fesolate 40mg/kg (group 7) did not show significant difference in number of proliferating cells when compared to group 3, as shown in Fig. 1g.

Table 2. Showing results of haemoglobin concentration, white blood cell count and bone marrow cell count

Groups	haemoglobin conc (g/dL)	White blood cell (x10^9/L)	bone marrow cell/hpf
1	14.75±0.38	9.51±0.19	461.50±12.17
2	6.17±0.29*	3.40±0.29*	232.00±43.73*
3	6.35±0.11*	2.80±0.31*	231.25±5.28*
4	9.07±0.37*,a	5.28±0.24*,a	366.25±4.31*,a
5	11.27±0.33*, a,b	7.14±0.24*,a,b	383.25±3.42*,a
6	14.44±0.53*,a,c	8.01±0.39a,c	442.75±12.43*,a,b
7	11.74±0.37*,a,b	6.90±0.22*, a, b	444.00±14.69*,a,b

** = p<0.05 vs Control; a = p<0.05 vs Docetaxel Alone, b= p<0.05 vs C+Docetaxel+Tan (LD)*

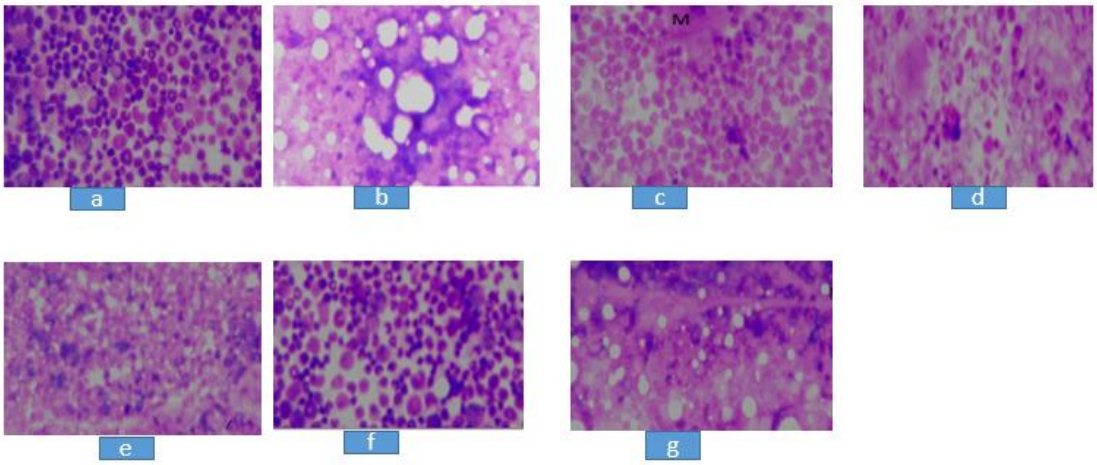

Fig. 1. Photomicrograph of bone marrow cytology smear (X400), stained with Giemsa stain, showing many nucleated cells (blue) with varying numbers. Near absence of nucleated cells noted in b. f showed large number of nucleated cells

4. DISCUSSION

Tannin fractionate of *Vitex doniana* leaf extract significantly increased the levels of the white blood cell count and haemoglobin concentration in this study. Tannin may have exerted these changes due to its antioxidant activity in scavenging free radicals, removing transition metals, and inhibition of pro-oxidative enzymes. This finding correlates to that of Stukelj and colleagues, who added tannin to the diets of experimental animals, and noted that there was no deleterious effect on the haematological parameters after 21 days of feeding [20]. Olafadehan et al., 2011 found that there were increase in the haemoglobin concentration, packed cell volume, white blood cell count of experimental animals fed with tannin-rich Pterocarpus erinaceus diet when compared with the blood parameters of those fed with pure diet [21]. Souza et al., in their study in 2020 suggested that tannin was responsible for the significant increase in the white blood cell count of experimental rats treated with extract from Saccharum officinarum for a period of 28 days [22].

This study shows that Tannin is protective to bone marrow during chemotherapy administration. Tannin probably exerts this effect because of its ability to inhibit lipid peroxidation and ability to scavenge free radicals important in cellular pro-oxidant states. This finding correlates with results of Xiong and colleagues in 2014, who observed that myelosuppression induced by cyclophosphamide 200mg/kg was reversed by tannins. They noted that tannins from Sanguisorbae radix significantly increase the numbers of white blood cells, red blood cells, and platelets of myelosuppression in the mice. And also accelerated bone marrow haemopoietic progenitor cells in the myelosuppression mice [23].

5. CONCLUSION

Tannin fractionates of *Vitex doniana* leaf have proven to have haemopoietic and anti-myelosuppressive effects. This fractionate can be used in combination with cytotoxic chemotherapeutic agents to prevent the unwanted effects of bone marrow suppression, anemia, and leukopenia that may be associated with use of chemotherapeutic agents. Most importantly, the purification of this fractionate of *Vitex doniana* leaf can enhance its use in clinical practice for treatment of anaemia, leukopenia, and in combination with other chemotherapeutic agents, as its purification may lead to better bioavailability and enhanced pharmacokinetics, with attendant improvement in health of patients.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical clearance for this study was obtained from The Ethical Committee, Faculty of Basic Medical Sciences, University of Nigeria, Enugu Campus.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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