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Myeloprotective activity of methanolic leaf extract of *Jatropha tanjorensis* in cyclophosphamide induced bone marrow suppression

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ABSTRACT

This study was designed to investigate the myeloprotective activity of methanolic leaf extract of Jatropha tanjorensis in cyclophosphamide induced bone marrow suppression in albino wistar rats. Myelosuppression was achieved by intraperitoneal injection of 3mg/kg of cyclophosphamide for 7 consecutive days. The extract was tested orally at 200 mg/kg and 400 mg/kg, given for 14 days, while Vitamin B complex (a hematinic) served as a reference drug. The degree of protection was determined by measuring the levels of hematological parameters such as: TWBC, RBC, PCV, HB and Reticulocyte count. Results obtained showed that both doses of the extract significantly ($p < 0.05$) increased the values of all the hematological indices estimated, which included: total white blood cells, red blood cells, packed cell volume, hemoglobin and reticulocyte count. The activity of the extract was dose – dependent and comparable to that of that reference drug. The results thus suggest that Jatropha tanjorensis may possess myeloprotective activity when administered orally in cyclophosphamide induced bone marrow suppression. This finding supports the folkloric use of the leaves of this plant for prevention and treatment of anaemia.

Key words: Cyclophosphamide, *Jatropha tanjorensis*, hematology, extract, myelosuppression.

INTRODUCTION

Myelosuppression is a condition in which the bone marrow activity is decreased, resulting in anaemia, thrombocytopenia and neutropenia. The consequences of the above are: fatigue, spontaneous severe bleeding and increased risk of potential fatal infections [1]. It is a serious side effect of certain drugs and the most serious adverse effect of cancer chemotherapy [2]. Conventionally, myelosuppression is managed by reduced dosing which unfortunately lowers the effectiveness of treatment [3]. Prophylactic use of granulocyte colony stimulating factor (G-CSF) has been shown to reduce the severity and duration of chemotherapy – induced neutropenia [4]. Transfer of drug resistant genes has also been demonstrated to protect the hematopoietic system from the toxicity of anticancer chemotherapy [5]. The above measures are not only expensive, but yield results that are unsatisfactory. In view of the exponential rise in the number of cancer patients, resulting in increased use of anticancer drugs, there is urgent need for affordable regimen that can protect the bone marrow against the damaging effects of anticancer drugs and other chemotherapeutic agents injurious to the bone marrow. *Jatropha tanjorensis* is one plant that is under investigation for its hematopoietic potential. Researchers have reported significant enhancement of hematological indices by the plant [6]. There is also documentation of its hypoglycaemic and antidiabetic activities [7]. Hypolipidaemic and antimicrobial effects of *Jatropha tanjorensis* have also been demonstrated [8][9]. The plant

belongs to the family *Euphorbiaceae* and is a common weed of field crops in the higher rain forest zones of West Africa. It is called " hospital too far" or "catholic vegetable" in Nigeria, where the leaves are consumed for the purpose of boosting blood level as well as a natural remedy for diabetes mellitus [10]. Preliminary phytochemical analysis of methanolic extract of *J. tanjorensis* revealed the presence of saponins, glycosides, flavonoids, alkaloids, anthroquinones and tannins [11].However this study investigates the myeloprotective activity of methanolic leaf extract of *Jatropha tanjorensis* in cyclophosphamide induced bone marrow suppression in albino rats.

MATERIALS AND METHODS

2.1 Plant materials (collection and preparation)

Fresh leaves of the plant were collected from its natural habitat and authenticated at the Department of Botany, Michael Okpara University of Agriculture, Umudike. The leaves were dried under mild sunlight, then reduced to coarse powder , using electric blender. Two hundred grams of the powder were exhaustively extracted with 80% methanol, using the method of cold maceration with intermittent shaking for 72 hours. Following filtration, the solvent was evaporated and the extract dried in hot air oven (40 ^{0}C) and stored in the refrigerator (4 ^{O}C) before use. Percentage yield was determined using the formula:

$$\% \text{ yield} = \frac{\text{weight of extract}}{\text{weight of plant material}} \times 100$$

2.2 Animals

Albino rats (120 – 160g) were procured from the laboratory animal house of the University of Nigeria, Nsukka. The animals were allowed two weeks for acclimatization and fed *ad libitum* with standard rat chow and clean drinking water. All animal experiments were carried out in accordance with NIH guidelines for care and use of laboratory animals.

2.3 Acute toxicity test

Twenty five albino rats of both sexes were randomly divided into 5 groups (*n = 5*). Group A received distilled water (10ml/kg) orally while groups B, C, D and E were treated with 500, 1000, 2000 and 4000mg/kg of methanolic extraxt of *Jatropha tanjorensis* (MEJ) *per os*. The animals were observed for 48 hours for signs of acute toxicity and death.

2.4 Effect of MEJ on cyclophosphamide – induced myelosuppression

Thirty albino wistar rats were randomly divided into 5 groups *(n = 6)* and treated as follows: group A served as normal untreated group. Group B received 3mg/ kg of cyclophosphamide (intraperitoneally) and 10ml/kg of distilled water *per os* for 7 days. Group C was given 3mg/kg of cyclophosphamide, i.p. and 5ml/kg vitamin B complex orally for 7 days. Group D received cyclophosphamide (3mg/kg) i.p. and 200mg/kg of MEJ orally for 7 days. Group E was given cyclophosphamide (3mg/kg) intraperitoneally and 400mg/kg of MEJ orally for 7 days. On the 7th day, injection of cyclophosphamide was terminated while administration extract and vitamin B complex continued till day 14. On the 15th day, blood samples were collected from each animal through the retro orbital plexus of the median canthus of the eyes, using heparinized capillary tubes into K3-EDTA (Tri-potassium ethylenediamine tetraacetic acid) bottles for hematological analysis, following standard methods [12].

2.5 Statistical analysis

Data generated from the study were analyzed using the one way analysis of variance (ANOVA) and the variant means separated using the least significant difference (LSD) of the groups. Significance was accepted at the level of $p < 0.05$.

RESULTS

3.1 Extraction and acute toxicity study

The extract gave a yield of 8.45% w/w dry matter. It was oily and dark green in color. The acute toxicity study recorded neither death nor sign of acute toxicity even at the highest dose tested (i.e. 4000mg/kg).

3.2 Effect of MEJ on some hematological parameters in cyclophosphamide – induced myelosuppression

In a dose – dependent manner, the methanolic extract of *J. tanjorensis* significantly ($p < 0.05$) increased the following hematological indices: total white blood cells, red blood cells, packed cell volume, hemoglobin and reticulocytes count, when compared with the negative control group (Table 1)

Table 1: Effect of MEJ on some hematological parameters in cyclophosphamide – induced myelosuppression

Groups	Hb (g/dl)	PCV (%)	RBC ($\times10^{12}$/L)	TWBC ($\times10^{9}$/L)	Reticulocyte (%)
A. untreated	11.68 ± 0.25*	41.13 ± 0.90*	6.63 ± 0.30*	7.30 ± 0.58*	2.08 ± 0.05*
B. Water + cp	6.10 ± 0.11	28.50 ± 0.35	3.68 ± 0.17	3.03 ± 0.08	0.73 ± 0.05
C. Vit. B co. + cp	9.70 ± 0.12*	37.75 ± 0.60*	5.73 ± 0.25*	6.33 ± 0.26*	1.50 ± 0.16*
D. MEJ 200 mg/kg +cp	6.85 ± 0.13*	33.25 ± 0.48*	4.73 ± 0.14*	4.55 ± 0.13*	1.03 ± 0.06*
E . MEJ 400 mg/kg + cp	7.85 ± 0.13*	36.63 ± 0.47*	5.23 ± 0.11*	4.98 ± 0.13*	1.25 ± 0.12*

** p > 0.05 when compared to the negative control.cp – cyclophosphamide; vit .B co. – vitamin B complex.*

DISCUSSION

Cyclophosphamide is a chemotherapeutic agent used to treat various types of cancer and some autoimmune diseases [13]. Being a Nitrogen mustard alkylating agent, it irreversibly attaches the alkyl group (CnH2n+1) to the guanine base of DNA, at number 7 nitrogen atom of the imidazole ring resulting in cell death [14]. One of the side effects of cyclophosphamide therapy is bone marrow suppression, resulting in low levels of all blood cell types in hematological analysis [15]. The drug was therefore used to induce myelosuppression in the experimental animals. From the results of the study there is significant increase in the values of all the hematological parameters (TWBC, RBC,PCV, Hb and Reticulocytes count) in the test groups when compared with the control. It could be that the extract offered some protection to the bone marrow against the cytotoxic effects of cyclophosphamide. It could also be that that *J. tanjorensis* exerts stimulatory effect on the hematopoietic activity of the bone marrow cells as evidenced by the significant high reticulocyte count in the test groups when compared with the control (Table 1). This result may justify the consumption of the leaves by the natives for boosting of blood. The acute toxicity study produced neither death nor acute toxicity sign even at the highest dose (4000mg/kg) thus, the extract was well tolerated by the animals at the doses tested.

CONCLUSION

Jatropha tanjorensis may possess principles that could protect the bone marrow against the suppressive effect of cyclophosphamide as evidenced by the significant ($p > 0.05$) increase in the hematolohical indices in the test animals when compare with the negative control. The leaves of *J. tanjorensis* may therefore serve as an adjunt to cancer chemotherapy as well as a potent regimen for prevention and treatment of anaemia.

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