Original Article

Myeloprotective activity of crude methanolic leaf extract of *Cassia occidentalis* in cyclophosphamide-induced bone marrow suppression in Wistar rats

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Abstract

Background: Myelosuppression is the most common dose-limiting side effect of chemotherapy. *Cassia occidentalis* plays a vital role in preventing health disorders, but its hematological effects have not been documented much. This study is designed to investigate the myeloprotective activity of the crude methanolic leaf extract of *C. occidentalis* in cyclophosphamide-induced bone marrow suppression.

Materials and Methods: Twenty-eight Wistar rats aged two to three months, weighing 120-170 g were used for the study. The rats were divided into four groups of seven rats each, labeled A to D. Groups A and B were administered with 3 mg/kg of cyclophosphamide intraperitoneally daily for three days to induce bone marrow suppression, after which groups B and C were orally fed with 250 mg/kg body weight of the crude leaf extract once daily for 14 days. Group D served as control without receiving the extract. On Day 15, blood samples (3.0 ml) were collected from each rat through the retro-orbital plexus of the median canthus into K_3-EDTA containers for hematological analysis using standard operative procedures. Data were analyzed with Pearson's correlation test and multivariate analysis of variance using Statistical Package for Social Sciences (SPSS) version 17 and results were expressed as mean ± SD. The level of significance was determined at 95% confidence level.

Results: Myelosuppression was achieved in Group A rats. Group B rats showed a significant increase in hemoglobin (Hb), hematocrit (Hct), and total white blood cell count (TWBC) compared with Group A. The Group C rats revealed a significant increase ($P < 0.05$) in Hb, Hct and TWBC when compared with control.

Conclusions: Crude methanolic leaf extract of *C. occidentalis* may possess myeloprotective properties when orally administered in cyclophosphamide-induced bone marrow suppression.

Key words: *Cassia occidentalis*, hematological parameters, leaf extract, myelosuppression

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INTRODUCTION

The *Cassia* spp. (family: *Fabaceae)* are erect, lightly branched leguminous trees and shrubs. Cassia shrubs are usually six to eight feet tall and are mostly annual, but some species are perennial. All *Cassia* spp. are toxic or poisonous, but *C. occidentalis* and *C. obtusifolia* are

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considered to be more toxic than others.[1] Although all parts of the plants are toxic, most poisoning occurs when animals eat the pods and beans, or are fed green-chop containing Cassia plants.[1]

Cassia occidentalis Linn *(C. occidentalis)* is a herbaceous species that is native to the Americans. It belongs to the family Fabaceae *(*Leguminosae*)* and subfamily Caesalpinioideae.[2]

The leaves of *C. occidentalis* are alternate, compound and pinnate, consisting of four to five pairs of leaflets widely spaced along a common stalk. The flowers are yellow and produced in loose clusters in the terminal leaf axils. The fruits of the plant are in the form of 'pods'-thin, flat, three to four inches long and pale green when they are tender and thick and dark green when they mature.[1]

The pods are slightly curved and with paler longitudinal stripes along the edges, with each pod containing around 50-60 small beans (seeds) together weighing 1.9-2.25 g. Each bean is about the size of a cumin seed, but shorter; the tender beans, which taste like peas, are green, soft and juicy.[1]

The roots, flowers, seeds and leaves of the species are used in herbal medicine throughout the world for a variety of purposes: As a laxative, expectorant, blood purifier, analgesic, antimalarial and relaxant and in wound healing.[3] *C. occidentalis* is used in several traditional medicines to cure various diseases, with antibacterial, antifungal, antidiabetic, anti-inflammatory, anticancer, antimutagenic, and hepatoprotective activities. Also, it has been used to treat snakebites[4] and as a potent abortifacient.[5] Cassia species are a good source of mucilage, flavonoids, anthraquinones, and polysaccharides. They also yield timber, dyes and tannins, fodder, vegetables, edible fruits, and seeds used as a substitute for coffee.[5]

Scientific studies have shown that *C. occidentalis* has an enormous biological potential and may have anti-inflammatory, antiplatelet, muscle relaxant, and antihemolytic activities and inhibit lipid peroxidation. These effects may occur probably as the species is rich in derivatives like anthraquinones.[6] Several studies have reported the isolation and structure elucidation of three new flavone glycosides from the methanolic extract of the seeds of *C. occidentalis*.[6] Leaf extracts of *C. occidentalis* can reduce the inflammatory effects of Bothrops snake (*Bothrops moojeni*) poison and accelerate the healing process.[7]

The use of many chemotherapy drugs lead to some degree of myelosuppression,[6] characterized by a decrease in bone marrow cellularity, frequency, and content of stem and progenitor cells.[8] Granulocyte-macrophage progenitors (CFU-GM) are the most important suppressed group among hematopoietic cells resulting in neutropenia.[9]

Oral administration of the crude extract of *C. occidentalis* as herbal remedy may affect blood cell production due to its flavanoid and phytosterol contents, which have been described as possible candidates that increase white blood cells in addition to compounds that might affect red cell production,[10] hence the need for this study.

We therefore tested the hypothesis that oral administration of the crude methanol leaf extract of *C. occidentalis* may affect the hematological parameters in Wistar rats with cyclophosphamide-induced bone marrow suppression, and that this effect might be in the form of increased production of the blood parameters, which will in turn cushion the effect of myelosuppression in the animals. This property could offer a possible way to cushion the effect of anticancer drug therapy on the myeloid cells and thus prevent myelosuppression during chemotherapy.

MATERIALS AND METHODS

The leaves of *C. occidentalis* were obtained from its natural habitat in Nigeria in the month of June (rainy season) and authenticated by the Botany department; University of Nigeria Nsukka and voucher specimens were kept in the herbarium for future reference.

Twenty-eight Wistar rats were purchased and housed in the Animal House of the College of Medicine, University of Nigeria Enugu Campus. The rats were kept together for two weeks to acclimatize and were fed with commercially available rat feed and had access to feed and water *ad libitum*.

The authors used whole plant extracts instead of standardized herbal extracts, because whole herbs contain all of the constituents of the plant and have been used for many years by many cultures. Also the full medicinal value of herbs is most likely due to their internal complexity and to interactions of the different components within the body, rather than to one of its specific components.[11] Therefore, it is doubtful that this type of standardized herbal extract can exhibit the same full spectrum of use as the whole herb.[11] Moreover, the whole plant extract is usually administered in the treatment of different medical conditions that have been attributed to *C. occidentalis*.

From the ground and shade dried *C. occidentalis* leaves, 100 g of the powder was extracted exhaustively

with methanol and the mixture was sieved. The remaining methanol in the extract was evaporated to get the concentrated crude extract which was reconstituted with 3% dimethyl sulfoxide (DMSO) and stored in the refrigerator until needed. The total yield of the methanol extract before reconstitution with DMSO was found to be 8.30%.

The twenty-eight Wistar rats were divided into four groups of seven rats per group, labeled A to D. Groups A and B were induced for bone marrow suppression with 3 mg/kg of cyclophosphamide intraperitoneally for three days after which groups B and C were orally fed with 250 mg/kg body weight of the crude methanolic leaf extract of *C. occidentalis* daily for 14 days. We chose a single dose (250 mg/kg) which is very safe considering that the LD 50 of the plant has been found to be 1600 mg/kg body weight in methanol crude extract,[12] since we did not evaluate the dose-dependent effect of the extract. Also, previous research stated that the extract causes degranulation of mast cells at 250 mg/kg. Group D served as control without receiving the extract. On Day 15, blood samples (3.0 ml) were collected from each rat through the retro-orbital plexus of the median canthus of the eyes with capillary tubes into tri-potassium ethylenediamine tetracetic acid (K_3-EDTA) containers for hematological analysis using standard operative procedures as described by Dacie and Lewis.[13]

Data were analyzed using Statistical Package for Social Sciences (SPSS) version 17. Associations between the different groups and significant differences were examined using Pearson correlation test and multivariate analysis of variance, at 95% confidence level, with the $P < 0.05$ being considered as significant.

RESULTS

Table 1 shows the mean and standard deviation (Mean ± SD) of some hematological parameters in treated and control rats. The results revealed that myelosuppression was achieved in group A rats that received cyclophosphamide alone. Group B rats that were also suppressed with cyclophosphamide and later received 250 mg/kg body weight extract revealed significant increases ($P < 0.05$) in hemoglobin (Hb), hematocrit (Hct) and total white blood cell count (TWBC) when compared with the myelosuppressed rats (group A), whereas a nonsignificant increase was recorded in Hb and Hct, with a significant decrease ($P < 0.05$) in TWBC when compared with control (Group D). The Group C rats that received 250 mg/kg body weight of the extract alone revealed a significant increase in Hb, Hct and TWBC when compared with control ($P < 0.05$). The blood films showed normocytic and normochromic red blood cells in all the groups despite the myelosuppression.

Table 1: Mean and standard deviation (Mean ± SD) of some hematological parameters of treated and control rats

Variables	Group A	Group B	Group C	Group D (control)
Hematocrit (l/l)	0.16±0.02*	0.35±1.0#	0.40±1.0*	0.34±0.08
Hemoglobin (g/dl)	5.6±0.98*	11.7±1.3#	12.8±0.35*	11.5±0.42
TWBC (x10^9)	1.43±0.5*	4.8±0.71*#	6.5±1.22*	5.8±0.35
Neutrophil (%)	19±1*	53±2.1#	65±2.05	60±3.0
Lymphocyte (%)	79±3*	45±1.0#	32±1.30	37±2.0
Monocyte (%)	1±0.21*	1±1.0	2±0.50	2±1.0
Eosinophil (%)	1±0.41	1±1.0	1±0.50	1±0.5

Group A received cyclophosphamide alone, Group B received cyclophosphamide and 250 mg/kg body weight extract, Group C received 250 mg/kg body weight extract alone, *$P < 0.05$ (statistically significant compared with control), *$P < 0.05$ (statistically significant compared with myelosuppressed rats)

DISCUSSION

In the current study, we tested the hypothesis that oral administration of the crude methanol extract of *C. occidentalis* might affect the hematological parameters in cyclophosphamide-induced bone marrow suppression, by increasing the blood cell production, which would cushion the effect of myelosuppression.

The results clearly revealed that myelosuppression was achieved in Group A rats that received cyclophosphamide alone. However Group B rats that were also suppressed with cyclophosphamide and later received 250 mg/kg body weight extract revealed significant increases ($P < 0.05$) in Hb, Hct and TWBC when compared with the myelosuppressed rats (Group A), whereas a nonsignificant increase was recorded in Hb and Hct, with a significant decrease ($P < 0.05$) in TWBC when compared with control (Group D), indicating that this extract may possess myeloprotective properties but the short duration of the study may have affected the result. The Group C rats that received the extract alone revealed significant increases in Hb, Hct and TWBC when compared with control ($P < 0.05$) indicating that this extract may probably possess properties that stimulate the bone marrow to produce more Hb and leucocytes when orally administered. This may be attributed to its anthraquinone and flavonoid content.

Anthraquinones are group of functionally diverse aromatic chemicals, structurally related to anthracene, with a parent structure 9, 10-dioxoanthracene. Anthraquinone compounds are used as laxatives mainly from their glycosidic derivatives and due to their wide therapeutic and pharmacological properties. Plant extracts containing anthraquinones are increasingly used in cosmetic, food, dye, and pharmaceutical industries. The root of *C. occidentalis*

contains 4.5% anthraquinone, which include 1.8 dihydroxy anthraquinone, emodin, quercetin, and a substance similar to rhein.[5] Anthraquinone derivatives show antioxidant properties and are and have been valued for their cathartic and detoxifying action,[5] a property that may have contributed to its myeloprotection in the present study.

While the leaves of *C. occidentalis* have been shown to be useful in disease treatment, its seeds have been implicated in a number of toxicological events in animals that consume them. Studies on rats fed a ration contaminated with *C. occidentalis* seeds at different doses showed histopathological and biochemical changes in muscles, liver, and central nervous system.[14] Histopathological study showed fiber degenerations in skeletal (tibial, pectoral and diaphragm) and cardiac muscles. In the liver parenchyma, vacuolar degeneration was observed and in the kidney, a mild necrosis in the proximal convoluted tubules. All these alterations occurred in a dose-dependent fashion. Moderate to severe degeneration and spongiosis were seen in the central nervous system, especially in cerebellum. Electron microscopy revealed mitochondrial lesions in all analyzed tissues.[12] Singh *et al*.[15] reported that *C. occidentalis* was used in the treatment of hemoglobin disorders in India. The leaves of *C. occidentalis* showed mainly the presence of flavonoids and anthraquinone glycosides such as rhein, emodin, physion obtusin, chrysophanol, and chryso obtusin.[15] These chemical constituents are responsible for the observed effects of the methanolic leaf extracts. Adequate care should be taken in administration of the seed extracts of the plant, whereas the leaf extracts with less reported toxic effects on animals should be consumed instead.

The blood films show normocytic and normochromic red blood cells in all the groups despite myelosuppression. This study shows that the methanol leaf extract of the plant, in addition to the already documented importance in disease treatment, also possesses possible myeloprotective ability in cyclophosphamide-induced myelosuppression in rats.

CONCLUSION

This study has demonstrated that oral administration of the crude methanolic leaf extract of *C. occidentalis* affects the bone marrow, thereby leading to leucocytosis and increased production of hemoglobin despite the myelosuppression induced by cyclophosphamide in Wister rats. These observed changes in the hematological parameters show the myeloprotective ability of the plant extract. Characterization of the leaf extracts and identification of the active components necessitate further studies.

A major limitation of the study is the inability to include graded doses and hence to determine the dose-dependent effect. This would have made the study more interesting although the authors chose a safe dose to administer, which gave the result.

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