

Myelo-protective Activity of Crude Methanolic Extract of Leaves of *Gongronema latifolium* in Cyclophosphamide-induced Myelo-suppression.

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Abstract: This study was designed to investigate the myelo-protective activity of crude methanolic extract of *Gongronema latifolium* leaves in cyclophosphamide-induced myelo-suppression. Wistar rats [n=30], aged 2 to 3 months, weighing 120 to 170 grams were acclimatized for two weeks and fed with commercially available rat feed and had access to water and feed *ad libitum*. They were divided into 5 groups [A to E]. Groups A, B and C were intraperitoneally-induced for myelo-suppression with 3mg/kg bodyweight of cyclophosphamide for 7 days. After induction, A served as cyclophosphamide-induced control, B received 150mg/kg, C received 250mg/kg and D [non-induced] received 250mg/kg [body-weight] of the extract orally for 14 days. Group E served as non-induced control. Blood samples [3.0 ml] were collected from each rat on days 8 and 15 through the retro orbital plexus of the median canthus into tri-potassium ethylene-diamine-tetracetic-acid containers for haematological analysis. Day 8, groups B and C revealed dosage-dependent significantly increased values compared to A [$p<0.05$] while D revealed non-significantly increased values compared to E. Day 15 groups B and C revealed dosage- and time-dependent significantly increased values compared to A while D revealed time-dependent significantly increased values compared to E. This suggests that this extract may possess both myelo-protective and haematopoietic effects when orally administered in cyclophosphamide-induced myelo-suppressed and non-induced Wistar rats.

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1. Introduction:

Herbal medicines are medicines derived from indigenous plants and is especially predominant in developing countries where modern Western medicine are often unavailable or is simply too expensive and this is particularly so in rural areas where they rely heavily on herbal preparations for the treatment of various diseases (WHO, 2002). The uses of many chemotherapy drugs lead to some degree of myelosuppression (Ozkan, *et al*, 2005). Myelo-suppression is characterized by the decrease in bone marrow cellularity, frequency and content of stem and progenitor cells. Granulocyte-macrophage progenitors (CFU-GM) are the most important suppressed group among haematopoietic cells resulting in neutropenia (Ozkan, *et al*, 2005).

Gongronema latifolium is a climbing perennial plant that belongs to the family of Asclepidaceae (Okafor and Ejiofor, 1996; Eleyinmi *et al*, 2006). It is a rainforest plant which has been traditionally used in the South-Eastern part of Nigeria for the management of diseases such as diabetes and high blood pressure (Ugochukwu, *et al* 2003). It is also used in the West

African sub-region for a number of medicinal and nutritional purposes such as spice and vegetable (Dalziel, 1955). The plant is traditionally used in the control of weight gain in lactating women and promotes fertility in women (Schneider, *et al*, 2003). *Gongronema latifolium* is used in the treatment of malaria, loss of appetite, cough, worm and dysentery (Agbo, *et al*, 2005). The plant is a good source of vitamins, minerals and protein (Okafor, 2005). Its phytochemical analysis reveals the presence of essential oil, saponin, alkaloids, minerals like calcium, phosphorous, magnesium, copper and potassium (Schneider, *et al*, 2003).

Much haematological effects have not been recorded on *Gongronema latifolium*. There is limited information on the myelo-protective activity of leaf extract of *Gongronema latifolium*. Due to this paucity in information and its numerous medicinal properties and uses, it becomes necessary to investigate the myelo-protective activities of crude methanolic extract of *Gongronema latifolium* leaves in cyclophosphamide-induced myelo-suppression in Wistar rats. This could offer a possible way to cushion

the effect of anti-cancer drug therapy on the myeloid cells and hence prevent myelo-suppression during the duration of therapy and beyond. It is believed that during usage of crude extract of *Gongronema latifolium* as an herbal remedy, that it may be either stimulating the bone marrow to produce more blood cells or it may be suppressing the bone marrow to cause anemia.

2. Materials and Methods:

Collection of Plant Materials: The leaves of *Gongronema latifolium* were obtained from its natural habitat in Enugu, Nigeria and authenticated by a Taxonomist in the Botany department; University of Nigeria Nsukka and a voucher specimen were kept in the herbarium for future reference.

Animals: Wistar rats (n=30) 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 have access to feed and water *ad libitum*.

Preparation of Extract: One hundred (100) grams of the powder from the grinded shade dried *Gongronema latifolium* leaves were extracted exhaustively with methanol and the mixture sieved. The remaining methanol in the extract was evaporated to get the concentrated crude extract which was reconstituted with 3% *Dimeyhylsulphoxide (DMSO)* and stored in the refrigerator until needed.

Experimental Design: Wistar rats (30) were divided into 5 groups (A to E). Groups A, B and C were induced intraperitoneally for myelo-suppression with 3mg/kg bodyweight [b.wt] of cyclophosphamide [CP] for 7 days. After induction A served as CP-

induced control (B received 150mg/kg, C received 250mg/kg, and D [non-induced] received 250mg/kg) [b.wt] of the extract orally for 14 days. Group E served as non-induced control. Blood samples (3.0 ml) were collected from each rat on days 8 and 15 through the retro orbital plexus of the median canthus into tri-potassium ethylene diamine tetracetic acid [K₃-EDTA] containers for haematological analysis (Haemoglobin [Hb], Haematocrit {Hct}, Red Blood Cell [RBC] and Total White Blood Cell [TWBC] using standard operative procedures as described by Dacie and Lewis (2006).

Statistical Analysis: The Statistical Package for Social Science (SPSS) computer software version 15 was used for data analysis. The results of the tests were analyzed using analysis of variance (ANOVA) and student's *t*-test at 95% confidence interval with *p* value of ≤ 0.05 been considered as significant.

3. Results:

The results of this study were expressed in tables 1 and 2. Table 1 shows the Day 8 (Protective Phase) mean and standard deviation (Mean \pm SD) of some Hematological parameters of extracts treated CP-induced, non-induced and control Wistar rats. Groups B and C revealed dosage-dependent significantly increased values compared to A while D revealed no significant increased values compared to E. Table 2 shows the Day 15 (Recovery Phase) mean and standard deviation (Mean \pm SD) of some Hematological parameters of extract treated CP-induced, non-induced and control Wistar rats. Groups B and C revealed dosage dependent significantly increased values compared to A while D revealed significantly increased values compared to E.

Table 1: Day 8 (Protective Phase) Mean and Standard Deviation (Mean \pm SD) of Some Hematological Parameters of Extract Treated CP-induced, Non-induced and Control Wistar Rats

Groups Variables	A CP-induced control	B CP-induced/ 150mg/kg b.wt Ext	C CP-induced/ 250mg/kg b.wt Ext	D Non-induced/250mg/kg b.wt Ext	E Non-induced control
Haemoglobin g/dl	5.5 \pm 1.2	8.6 \pm 1*	11.3 \pm 0.5	12.1 \pm 1	11.5 \pm 0.8
Haematocrit l/l	0.15 \pm 0.01	0.24 \pm 0.02*	0.32 \pm 0.02	0.35 \pm 0.01	0.33 \pm 0.02
Red Blood Cell $\times 10^{12}/l$	2.05 \pm 0.1	3.1 \pm 0.2*	3.8 \pm 0.1	4.5 \pm 0.2	4.3 \pm 0.2
Total WBC $\times 10^9/l$	1.5 \pm 1.0	3.8 \pm 0.5*	4.5 \pm 0.35	5.2 \pm 1.5	5.1 \pm 1.0

Table 2: Day 15 (Recovery Phase) Mean and Standard Deviation (Mean \pm SD) of Some Hematological Parameters of Extract Treated CP-induced, Non-induced and Control Wistar Rats

Groups Variables	A CP-induced control	B CP-induced/ 150mg/kg b.wt Ext	C CP-induced/ 250mg/kg b.wt Ext	D Non-induced/ 250mg/kg b.wt Ext	E Non-induced control
Haemoglobin g/dl	7.5 \pm 1.2	11.2 \pm 1.5*	13.0 \pm 0.5*	13.6 \pm 0.5*	11.5 \pm 0.8
Haematocrit l/l	0.22 \pm 0.01	0.31 \pm 0.02*	0.37 \pm 0.02*	0.39 \pm 0.01*	0.33 \pm 0.02
Red Blood Cell $\times 10^{12}/l$	3.5 \pm 0.1	3.9 \pm 0.2	4.2 \pm 0.1	4.8 \pm 0.2*	4.3 \pm 0.2
Total WBC $\times 10^9/l$	2.2 \pm 1.0	4.6 \pm 0.5	5.3 \pm 0.5*	6.1 \pm 1.5*	5.1 \pm 1.0

Key: * $p < 0.05$

4. Discussions:

Gongronema latifolium is an edible plant found in the tropical rainforest and is frequently used for treatment of various disease conditions like malaria, loss of appetite, cough, worm and dysentary (Agbo, *et al*, 2005). It is also used in the management of diabetes and high blood pressure (Ugochukwu, *et al* 2003). The plant is a good source of vitamins, minerals and protein (Okafor, 2005). Its phytochemical analysis reveals the presence of essential oil, saponin, alkaloids, minerals like calcium, phosphorous, magnesium, copper and potassium (Schneider, *et al*, 2003).

Much haematological findings have not been reported on *Gongronema latifolium*. Due to the paucity of haematological informations of *Gongronema latifolium* in the science literature and its numerous medicinal properties and uses, it becomes necessary to investigate the myelo-protective activity of crude methanolic extract of leaves of *Gongronema latifolium* in cyclophosphamide-induced myelo-suppression in Wistar rats. Cyclophosphamide have been known to act by slowing or stopping cell growth (Shanafelt, *et al*, 2007).

During the protective phase, (Day 8), the CP-induced groups B and C revealed dosage-dependent significantly increased values compared to A (cyclophosphamide-induced control) indicating myelo-protective effects of the extract while the non-induced group D revealed non-significantly increased values compared to E (non-induced control) probably due to the duration of the study indicating time-dependent haematopoietic effect of the extract. During the recovery phase, (Day 15), groups B and C revealed significantly increased values compared to A indicating dosage- and time-dependent myelo-protective effects while C and D revealed significantly increased values compared to E also indicating dosage- and time-dependent myelo-protective and haemopoietic effects of the crude methanolic extract

of leaves of *Gongronema latifolium*. However, in the recovery phase, (Day 15), group A showed non-significantly increased values compared to group A of the protective phase (Day 8), thereby demonstrating recovery from the myelo-suppressive actions of the cyclophosphamide. The observed dosage- and time-dependent myelo-protective and haemopoietic effects of this extract agrees with the findings of previous researchers which states that this extract posses both haematopoietic and anti microbial activities (Eleyinmi, *et al*, 2006).

The continuous increase in the haematological parameters in groups C (CP-induced/ 250mg/kg) and D (non-induced/ 250mg/kg) can be attributed to the contents of the leaves as shown by the phytochemical studies. The phytochemical constituents of this extract include essential oil, saponin, alkaloids, and minerals like calcium, phosphorous, magnesium, copper and potassium (Schneider, *et al*, 2003). These constituents might have contributed to the myelo-protective and haematopoietic activities as mainly observed in the higher CP dose and non-induced rats.

In the conclusion, this study have demonstrated that ingestion of the crude methanolic extract of the leaves of *Gongronema latifolium* possibly affects the bone marrow, thereby leading to myelo-protection and haematopoiesis in CP-induced myelo-suppressed Wistar rats and in non-induced Wistar rats. However, further studies, needs to be carried out in order to characterize the leaf extract and hence, find out the active components and use to formulate myelo-protective drug that will be similar to the existing myelo-protective drugs like Mpl-ligand which reduces haematopoietic toxicity after a myelo-suppressive insult (Neelis, *et al*, 1998). If such herbal myelo-protective drug is formulated, it will be less expensive against the existing costly ones in our locality, thereby help in alleviating the living condition of the concerned people.

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