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EFFECTS OF XYLOPIA AETHIOPICA EXTRACT AND MELATONIN ON PLATELETS AND COAGULATION PROFILE OF CYCLOPHOSPHAMIDE INTOXICATED WISTAR RATS

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ABSTRACT

The study was done to evaluate the effects of *Xylopiaaethiopica* and melatonin on platelets and coagulation profile in cyclophosphamide intoxicated adult wistar rats. Pods of *Xylopiaaethiopica* were purchased from Orie-Ugba vegetable market, Umuahia North Local Government Area, Abia State, Nigeria. One hundred and ninety five matured wistar albino rats were used for the studies. Results were expressed as means ± standard error of mean (SEM). Statistical analysis was done using one-way analysis of variance (ANOVA). Significant differences were assessed at 95% level of significance between control and treated groups using Duncan and LSD (Post Hoc) tests. P values less than 0.05 were considered significant. Computer software package, SPSS version 21 was employedIn weeks one, two and three, there was significant decrease in platelet in all the treatment groups compared with control. Plateletresults varied significantly from week one to three in all the treatment groups except group 13. The results showed thatin weeks one and two, there was a dose dependent decrease in platelet count of rats treated with 10, 30 and 50 mg/kg of cyclophosphamide. Treatment with 400mg *Xylopiaaethiopica* alone and in combination with 0.5mg melatonin significantly increased the platelet counts of rats exposed to 10mg cyclophosphamide. Platelet counts of rats treated with 400mg *Xylopiaaethiopica* and 0.5mg melatonin weresignificantly higher than that of 400mg *Xylopiaaethiopica* alone in week two but not statistically different in week one. Similarly, treatment with 400mg *Xylopiaaethiopica* alone and in combination with 0.5mg melatonin significantly increased the platelet counts of rats exposed to 30mg and 50mg cyclophosphamide respectively.

Platelet counts of rats treated with 400mg *Xylopiaaethiopica* alone were significantly higher than that of 400mg *Xylopiaaethiopica* and 0.5mg melatonin in week two in both rats exposed to 30mg and 50mg cyclophosphamide respectively. The platelet counts of rats exposed to 30mg cyclophosphamide and treated with 400mg *Xylopiaaethiopica* alone were not statistically different from that of its combination with 0.5mg melatonin in week one. In week three, treatment with 400mg *Xylopiaaethiopica* alone and in combination with 0.5mg melatonin significantly increase the platelet counts of rats exposed to 10mg cyclophosphamide.

In weeks one, two and three, there were significant increases in PTresults in all the treatment groups compared with control. PTresults varied significantly from week one to three in all the treatment except group 13. In week one, PT of rats exposed to 10 mg cyclophosphamide and treated with 400 mg *Xylopiaaethiopica* alone and in combination with 0.5 mg melatonin were significantly different from that of rats exposed to 10 mg cyclophosphamide alone.PTresults of rats treated with 400mg *Xylopiaaethiopica* and 0.5mg melatonin was not significantly different from the PTresults of rats exposed to 10mg cyclophosphamide and treated with 400 mg *Xylopiaaethiopica* alone. Similarly, PT results of rats treated with 400mg *Xylopiaaethiopica* alone and in combination with 0.5 mg melatonin were significantly decreased compared to PTresults of rats exposed to 30mg and 50mg cyclophosphamide respectively. By week two, PT results of rats exposed to 10mg cyclophosphamide and treated with 400 mg *Xylopiaaethiopica* alone and in combination with 0.5 mg melatonin were significantly different from rats treated with 10 mg cyclophosphamide alone. PT results of rats exposed to 30 mg and 50mg cyclophosphamide respectively and treated with 400mg *Xylopiaaethiopica* singly and in combination were significantly higher than that of the rats treated with 30mg and 50mg cyclophosphamide singly. PTresults of rats exposed to 30 mg cyclophosphamide and treated with 400 mg *Xylopiaaethiopica* singly was significantly different from rats exposed to 30mg cyclophosphamide and treated with 400 mg *Xylopiaaethiopica* and 0.5 mg melatonin. In week three, PTresults of rats exposed to 10mg cyclophosphamide treated with 400 mg *Xylopiaaethiopica* alone and in combination with 0.5 mg melatonin were significantly different from rats treated with 10mg cyclophosphamide.

In weeks one, two and three, there was significant increase in APTT results in all the treatment groups compared with control. APTT resultsvaried significantly from week one to three in all the treatment except group 13.In weeks one and two, there was a dose dependent increase in APTT results of rats treated with 10, 30 and 50 mg/kg of cyclophosphamide. In weeks one, two and three, treatment with 400mg *Xylopiaaethiopica* alone and in combination with 0.5mg melatonin significantly decrease the APT resultsof rats exposed to 10mg cyclophosphamide. Treatment with 0.5mg of melatonin combined with 400mg *Xylopiaaethiopica* decreases APTT results of 10mg cyclophosphamide exposed rats compared with APTT results of rats treated with 400mg *Xylopiaaethiopica* alone in week one but not so in week three. Similarly treatment with 400mg *Xylopiaaethiopica* alone and in combination with 0.5mg melatonin significantly decrease the APTT results of rats exposed to 30mg and 50mg cyclophosphamide respectively. Treatment with 400mg *Xylopiaaethiopica* alone decreases APTT results of 30mg and 50mg cyclophosphamide exposed rats compared with APTT results of rats treated with 0.5mg of melatonin combined with 400mg *Xylopiaaethiopica*.

KEYWORDS: *Xylopiaaethiopica*, melatonin, platelets, coagulation profile, cyclophosphamide intoxicatedwistar rats.

INTRODUCTION

Cyclophosphamide has been in use clinically to treat a wide range of cancers including malignant lymphomas, myeloma, leukaemia, mycosis, fungoides, neuroblastoma, adenocarcinoma, retinoblastoma, and breast carcinoma (Mohammed *et al*., 2017). Other clinical uses for cyclophosphamide can be seen in immunosuppressive therapy following organ transplants or as a treatment for autoimmune disorders such as rheumatoid arthritis, Wegener's granulomatosis, and nephritic syndrome in children (Chabner*et al*., 2001).The use of cyclophosphamide is however, limited by its toxicity. Some of the adverse effects may include alopecia, nausea, vomiting, thrombocytopenia, mucosal ulcerations, transverse striations in the nails, brief spells of dizziness, increased skin pigmentation, pulmonary fibrosis, leukopenia, facial abrasion, haematuria, diarrhoea, haemorrhagic cystitis, and petechial haemorrhage in lungs and small bowel (Gitanjah *et al*., 2017), but negative effects on the haematological systemhave been observed especially in leucocyte and platelet levels (Azevedo*et al*, 2007).

The hormone Melatonin is the main neuroendocrine secretary product of the pineal gland in animals and an evolutionary ancient derivative of serotonin with hormonal properties (slominski*et al*.,2018). It is also produced in plants where it functions as a first line of defence against oxidative stress (Tan *et al*., 2012).Previous studies showed that melatonin is preferentially localized inside the nucleus and can protect nuclear DNA from oxidative damage by interacting with double stranded DNA thereby promoting its stability. More so, it′s powerful antioxidant action acts either directly on free radical species or by modulating the gene expression of antioxidant enzymes such as glutathione peroxidase, catalase and superoxide dismutase. This antioxidant effect of melatonin involves DNA repair, and can repair the oxidation induced by the guanosine (G•) radical (Ferreira *et al*., 2013). It can protect tissues from the oxidative damage caused by glutathione depletion and ischemia-reperfusion injury. Apart from its antioxidant property, melatonin is a potential antitumor agent. Therefore, studying of the effects of melatonin in chemotherapy seems as an interesting area of investigation.

Xylopiaaethiopica, a shrub locally referred to as Ethiopian pepper, Negro pepper, Guinean pepper, Senegal pepper, Kili pepper and spice tree in the savannazone and coastal regions of Africa is amongst these plants with great therapeutic potential. It is an angiosperm belonging to the family Annonaceae (Obodo*et al*., 2013), and is among the species that thrive in the evergreen rain forests of tropical and subtropical Africa which matures into a slim, tall tree of approximately 60 cm in diameter and up to 30m high with a straight stem having a slightly stripped or smooth bark. *Xylopiaaethiopica* is used in the treatment of cough, biliousness, bronchitis, rheumatism, dysentery, malaria, uterine fibroid, amenorrhea, boils, sores, wounds and cuts among others (fetse*et al*., 2016). According to Obodo*et al*. (2013), The seeds of *Xylopiaaethiopica* have been shown to contain bitter chemical constituents like alkaloids, glycosides, saponnis, tannins, sterols, carbohydrate, protein, free fatty acids, mucilage's and acidic compounds; some of which might be responsible for its documented medicinal and pharmacological properties like anti-inflammatory, cytotoxic, hypoglycaemic and antioxidant effects.

Alteration in the integrity of blood leads to loss of blood and can be life threatening as blood is a necessary components of animal body. The body tends to protect itself from this life threatening exsanguination by converting the blood from its liquid state to a solid state in a process known as blood clotting or coagulation. This formation of a clot is often referred to as secondary haemostasis and it usually involves two main pathways namely extrinsic and intrinsic pathways that make use of clotting factors. Estimation of coagulation tests like prothrombin time ,activated partial thromboplastin time etc. are developed to diagnose disorders of coagulation which can lead to an increased bleeding (haemorrhage) or obstructive clotting (thrombosis) (Xiangqun, *et al*, 2014). For this study, the cyclophosphamide was chosen because it is one of the most frequently used antitumor agents in clinical practice and also its association with rapidly killing of dividing cells in the body. Considering the above, this present study was designed to evaluate the effects of *Xylopiaaethiopica* and melatonin on platelets and coagulation profile, in cyclophosphamide intoxicated wistar rats.

AIM

The study was done to evaluate the effects of *Xylopiaaethiopica* and melatonin on plateletsand coagulation profile (PT and APTT) in cyclophosphamide intoxicated adult wistar rats.

MATERIALS AND METHODS

Collection of plant materials and authentication: Pods of *Xylopiaaethiopica* were purchased from OrieUgba vegetable market, Umuahia North Local Government Area, Abia State, Nigeria, and were taken to the Department of Forestry and Environmental Management, Michael Okpara University of Agriculture, Umudike where they were identified by a botanist/forest manager. Voucher number MOUAU/VPP/18/012 was assigned to a specimen sampleof the pods which was deposited in the herbarium of the Department.

Preparation of plant extracts: Extract of the fruit pods was prepared in accordance with the Soxhlet method described by Jensen, (2007). The plant materials were subjected to further drying under shade for 14 days and were pulverized into powder in a manual blender powered by a Honda petrol engine. One hundred grams of the powdered sample was introduced into the extraction chamber of the soxhlet extractor and

extraction was carried out with ethanol as solvent. Temperature was maintained at 65^{0}C throughout the extraction period of 48 hours. At the end of the period, the extract in solution was dried in a hot air oven at 40^{o}C to obtain a dry dark oily extract. The weight of the extract was taken and percentage yield was calculated using the formular:

$$\% \text{ yield} = \frac{X}{Q} \times \frac{100}{1}$$

Where X = weight of dried extract and Q = weight of powdered plant material before extraction (100g) (Bandiola, 2018).

Animals used for study: One hundred and ninety five matured wistar albino rats were used for the studies. Of the number, 30 were used for the acute toxicity evaluation of the extract, 35 for acute toxicity study of cyclophosphamide and 130 were used for the main study. The rats were kept in aluminumcages and allowed to acclimatize for two weeks to allow for proper adaptation to the environment and living conditions. They were allowed access to feed (Vital feed, Nigeria) and water ad libitum but were starved for 12 hours prior to commencement of any experiment. All animal experiments were carried out in compliance with NIH guidelines for Care and Use of Laboratory Animals (OECD, 2001). All experiments were carried out in the Physiology Laboratory of the Department of Physiology and Pharmacology, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Nigeria.

Experimental design: The rats (130 in number) were assigned to 13 groups of 10 rats each and were treated according to the order below:

Group I: 10 mg/kg Cyclophosphamide, Food and water
Group II: 10mg/kg Cyclophosphamide, 400 mg/kg Extract, Food and water
Group III: 10mg/kg Cyclophosphamide, 0.5 mg/kg Melatonin, Food and water
Group IV: 10mg/kg Cyclophosphamide, 400mg/kg Extract, 0.5mg/kg Melatonin, Food and water
Group V: 30 mg/kg Cyclophosphamide, Food and water
Group VI: 30 mg/kg Cyclophosphamide, 400mg/kg Extract, Food and water
Group VII: 30mg/kg Cyclophosphamide, 0.5mg/kg Melatonin, Food and water
Group VIII: 30mg/kg Cyclophosphamide + 400mg/kg Extract, 0.5mg/kg Melatonin, Food and water
Group IX: 50 mg/kg Cyclophosphamide, Food and water
Group X: 50 mg/kg Cyclophosphamide, 400 mg/kg Extract, Food and water
Group XI: 50mg/kg Cyclophosphamide, 0.5mg/kg Melatonin, Food and water
Group XII: 50mg/kg Cyclophosphamide, 400mg/kg Extract, 0.5mg/kg Melatonin, Food and water
Group XIII: Food and water only

Treatments were done daily via the oral route for twenty one (21) days. Three animals were sacrificed in each group for blood collection by cardiac puncture into EDTA and sodium citrate bottles for platelet estimation and coagulation studies.

Haematological studies: Platelets (PLT) counts were determined using an Automated Haematology Analyser (Mindray company, China following the procedures by the producer.

Determination of activated partialthromboplastin time (APTT) using authomated kl340 coagulation analyser: Procedure; After gentle swirling of the reagent vials, enough volume of reagent 1 ($CaCl_2$) was pre-warmed for immediate use in a clean and dry plastic tube maintained at 37^{0}C. About 100µl of test plasma waspipetted into a test cuvette at 30^{0}C. About 100µl of pre warmed reagent 2 (APTT Reagent) was added to the cuvette. The mixture was well mixed and incubated at 37^{0}C for 3 minutes before forcibly pipetting 100µl of pre-warmed reagent 1 into the test cuvette while starting the stop watch at the same time to note time in seconds it takes for blood to clot.This time in seconds is recorded as the APTT.

Determination of prothrombin time (PT) using authomated kl340 coagulation analyser: Procedure**;** Prothrombin reagent was dispensed into a thoroughly clean and dry plastic tube for immediate use and prewarmed at 37^{0}C for 10 minutes. About 100 µl of test plasma was introduced into a test cuvette at 37^{0}C and incubated for 3 minutes before adding forcibly 200µl of the pre-warmed prothrombin reagent while starting the stop watch at the same time to record the time in seconds it takes for blood to clot. This time in seconds is recorded as the PT.

Statistical analysis: Results were expressed as mean ± standard error of mean (SEM). Statistical analysis was done using one way analysis of variance (ANOVA). Significant differences were assessed at 95% level of significance between control and treated groups using Duncan and LSD (Post Hoc) tests. P values less than 0.05 were considered significant. Computer software package, SPSS version 21 was employed.

Result of effects of *Xylopiaaethiopica* extract and Melatonin on Platelet of Cyclophosphamide intoxicated wistar rats

In weeks one, two and three, there was significant decrease in platelet counts in all the treatment groups compared with control.

In weeks one and two, there was a dose dependent decrease in platelet counts of rats treated with 10, 30 and 50 mg/kg of cyclophosphamide. Treatment with 400mg *Xylopiaaethiopica* alone and in combination with 0.5mg melatonin significantly increased the platelet counts of rats exposed to 10mg cyclophosphamide. Platelet counts of rats treated with 400mg *Xylopiaaethiopica* and 0.5mg melatonin were significantly higher than that of 400mg

Xylopiaaethiopica alone in week two but not statistically different in week one. Similarly, treatment with 400mg *Xylopiaaethiopica* alone and in combination with 0.5mg melatonin significantly increased the platelet counts of rats exposed to 30mg and 50mg cyclophosphamide respectively. Platelet counts of rats treated with 400mg *Xylopiaaethiopica* alone were significantly higher than that of 400mg *Xylopiaaethiopica* and 0.5mg melatonin in week two in both rats exposed to 30mg and 50mg cyclophosphamide respectively. The platelet counts of rats exposed to 30mg cyclophosphamide and treated with 400mg *Xylopiaaethiopica* alone were not statistically different from that of its combination with 0.5mg melatonin in week one.

In week three, treatment with 400mg *Xylopiaaethiopica* alone and in combination with 0.5mg melatonin significantly increase the platelet counts of rats exposed to 10mg cyclophosphamide.

Table 1: Effects of *Xylopiaaethiopica* extract and Melatonin on Platelet (PLT) of Cyclophosphamideintoxicated wistar rats.

Treatment	Platelet		
	Week 1 (Wk1)	Week 2 (Wk2)	Week 3 (Wk3)
10mg Cyclophosphamide	391±1.1^{bc}	365±0.6^{e}	319±0.5^{a}
10mg Cyclophosphamide+ 400mg *Xylopiaaethiopica*	495±1.7^{ef}	431±0.6^{f}	358±0.6^{c}
10mg Cyclophosphamide + 0.5mg Melatonin	429±0.6^{d}	373±0..5^{e}	
10mg Cyclophosphamide+ 400mg *Xylopiaaethiopica* + 0.5mg Melatonin	499±0.6^{ef}	444±1.7^{g}	327±0.6^{b}
30mg Cyclophosphamide	385±1.2^{b}	282±0.5^{b}	
30mg Cyclophosphamide+ 400mg *Xylopiaaethiopica*	443±2.3^{d}	322±0.5^{d}	
30mg Cyclophosphamide + 0.5mg Melatonin	387±3.5^{b}	290±0.5^{bc}	
30mg Cyclophosphamide+ 400mg *Xylopiaaethiopica* + 0.5mg Melatonin	444±1.7^{d}	310±0.5^{c}	
50mg Cyclophosphamide	300±2.9^{a}	265±0.6^{a}	
50mg Cyclophosphamide+ 400mg *Xylopiaaethiopica*	390±1.2^{bc}	310±0.5^{c}	
50mg Cyclophosphamide + 0.5mg Melatonin	304±1.1^{a}	269±0.6^{a}	
50mg Cyclophosphamide+ 400mg *Xylopiaaethiopica* + 0.5mg Melatonin	406±1.2^{c}	282±0.5^{b}	
Control	586±1.7^{g}	564±2.3^{h}	583±2.3^{d}

Result of effects of *Xylopiaaethiopica* extract and Melatonin onProthrombin Time(PT) in seconds (s) of Cyclophosphamide intoxicated wistar rats

In weeks one, two and three, there were significant increases in PT results in all the treatment groups compared with control. PT results varied significantly from week one to three in all the treatment except group 13.

In week one, PT results of rats exposed to 10mg cyclophosphamide and treated with 40 mg *Xylopiaaethiopica alone* and in combination with 0.5mg melatonin were significantly different from that of rats exposed to 10mg cyclophosphamide alone. PT results of rats treated with 400mg *Xylopiaaethiopica* and 0.5mg melatonin was not significantly different from the PT results of rats exposed to 10mg cyclophosphamide and treated with 400mg *Xylopiaaethiopica alone*. Similarly, PT results of rats treated with 400mg *Xylopiaaethiopica* alone and in combination with 0.5 mg melatonin were significantly decreased compared to PT results of rats exposed to 30mg and 50mg cyclophosphamide respectively.

By week two, PT results of rats exposed to 10mg cyclophosphamide and treated with 400mg *Xylopiaaethiopica* alone and in combination with 0.5mg melatonin were significantly different from rats treated with 10mg cyclophosphamide alone. PT results of rats exposed to 30mg and 50mg cyclophosphamide and treated with 400mg *Xylopiaaethiopica* singly and in combination were significantly higher than that of the rats treated with 30mg and 50mg cyclophosphamide singly. PT of rats exposed to 30mg cyclophosphamide

and treated with 400mg *Xylopiaaethiopica* singly was significantly different from rats exposed to30mg cyclophosphamide and treated with400 mg *Xylopiaaethiopica* and 0.5mg melatonin. In week three, PT of rats exposed to 10mg cyclophosphamide treated with 400 mg *Xylopiaaethiopica alone* and in combination with 0.5mg melatonin were significantly different from rats treated with 10mg cyclophosphamide.

Table 2: Result of effects of *Xylopiaaethiopica* extract and Melatonin on PT (s) of Cyclophosphamide intoxicated wistar rats

Treatment	PT (s)		
	Week 1 (Wk1)	Week 2 (Wk2)	Week 3 (Wk3)
10mg Cyclophosphamide	18.0±0.06^{d}	19.0±0.12^{f}	20.5±0.06^{d}
10mg Cyclophosphamide+ 400mg *Xylopiaaethiopica*	14.9±0.35^{b}	15.9±0.17^{c}	17.1±0.69^{b}
10mg Cyclophosphamide + 0.5mg Melatonin	17.5±0.06^{cd}	18.8±0.17^{f}	
10mg Cyclophosphamide + 400mg *Xylopiaaethiopica* + 0.5mg Melatonin	14.5±0.12^{ab}	15.1±0.12^{b}	18.7±0.12^{c}
30mg Cyclophosphamide	18.9±0.12^{e}	20.8±0.23^{g}	
30mg Cyclophosphamide + 400mg *Xylopiaaethiopica*	15.1±0.12^{b}	17.4±0. 69^{d}	
30mg Cyclophosphamide + 0.5mg Melatonin	18.4±0.17^{ed}	20.7±0.06^{g}	
30mg Cyclophosphamide + 400mg *Xylopiaaethiopica* + 0.5mg Melatonin	14.1±0.35^{a}	18.1±0. 23^{f}	
50mg Cyclophosphamide	19.7±0.06^{f}	21.9±0.12^{h}	
50mg Cyclophosphamide + 400mg *Xylopiaaethiopica*	18.1±0.17^{d}	19.7±0.06^{fg}	
50mg Cyclophosphamide + 0.5mg Melatonin	19.4±0.12^{ef}	21.5±0.12^{h}	
50mg Cyclophosphamide + 400mg *Xylopiaaethiopica* + 0.5mg Melatonin	18±0.17^{d}	20.1±0.12^{g}	
Control	13.8±0.06^{a}	14.3±0.35^{a}	14.4±0.17^{a}

Result of effects of *Xylopiaaethiopica* extract and Melatonin on APTT (s) of Cyclophosphamide intoxicated wistar rats

In weeks one, two and three, there was significant increase in APTT results in all the treatment groups compared with control. APTT results varied significantly from week one to three in all the treatment except group 13.

In weeks one and two, there was a dose dependent increase in APTT results of rats treated with 10, 30 and 50 mg/kg of cyclophosphamide.

In weeksone, two and three, treatment with 400mg *Xylopiaaethiopica alone* and in combination with 0.5mg melatonin significantly decrease the APTT results of rats exposed to 10mg cyclophosphamide.

Treatment with 0.5mg of melatonin combined with 400mg *Xylopiaaethiopica* decreases APTT results of 10mg cyclophosphamide exposed rats compared with APTT results of rats treated with400mg *Xylopiaaethiopica alone* in week one but not so in week three. Similarly treatment with 400mg *Xylopiaaethiopica alone* and in combination with 0.5mg melatonin significantly decrease the APTT results of rats exposed to 30mg and 50mg cyclophosphamide respectively. Treatment with 400mg *Xylopiaaethiopica* alone decreases APTT results of 30mg and 50mg cyclophosphamide exposed rats compared with APTT results of rats treated with 0.5mg of melatonin combined with 400mg *Xylopiaaethiopica*.

Table 3: Effects of *Xylopiaaethiopica* extract and Melatonin on APTT (s) of Cyclophosphamide intoxicated wistarrats.

Treatment	AVERAGE APTT (s)		
	Week 1 (Wk1)	Week 2 (Wk2)	Week 3 (Wk3)
10mg Cyclophosphamide	35.3±0.17^{de}	38.4±0.06 ^{d}	40.9±0.09 ^{d}
10mg Cyclophosphamide + 400mg *Xylopiaaethiopica*	32±0.58 ^{c}	34.4±0.06 ^{b}	37.5±0.17^{b}
10mg Cyclophosphamide + 0.5mg Melatonin	34.5±0.12 ^{d}	37.5±0.23^{cd}	
10mg Cyclophosphamide+ 400mg *Xylopiaaethiopica* + 0.5mg Melatonin	30.3±0.06 ^{b}	35±0.06^{bc}	39.2±0. 23^{c}
30mg Cyclophosphamide	37.5±0.06 ^{f}	40.6±0.06 ^{f}	
30mg Cyclophosphamide +400mg *Xylopiaaethiopica*	34.4±0.12^{d}	38.3±0.06^{d}	
30mg Cyclophosphamide + 0.5mg Melatonin	37±0.23^{f}	39.3±0.23^{ef}	
30mgCyclophoshamide+400mg *Xylopiaaethiopica* + 0.5mg Melatonin	35.9±0.06^{e}	38.7±0.06^{de}	
50mg Cyclophosphamide	44.1±0.06^{hi}	50.6±0.17^{i}	
50mg Cyclophosphamide +400mg *Xylopiaaethiopica*	36.4±0.35^{ef}	45.3±0.12 ^{g}	
50mg Cyclophosphamide + 0.5mg Melatonin	43.2±0.64 ^{h}	50.4±0.17^{i}	
50mg Cyclophosphamide +400mg *Xylopiaaethiopica* + 0.5mg Melatonin	39.1±0.23 ^{g}	48.4±0.06^{h}	
Control	29.3±0.35 ^{a}	29±0.12 ^{a}	29.07±0.03 ^{a}

DISCUSSION

The fall in platelets counts following cyclophosphamide(CP) administration in this studysuggested that thrombocytopenia may have occurred due to the cytotoxic effects of CP (Friken and Barnes, 1988; Ukpo et al., 2017). CP-induced thrombocytopenia (Glaser and Kiecolt-Glaser 2005) can increase the PLT destruction and/or reduce the PLTs production in bone marrow Hackett 2003. Bone marrow is the organ most affected by immunosuppressant. Loss of stem cells and inability of bone marrow to regenerate new blood cells will result in reduction of cells like thrombocytopenia. Our results also showed a significant increase in platelet results of rats treated with *Xylopiaaethiopica* (XA) and melatonin and a possible synergistic effect. This in effect agrees with the countering effect of XA on the toxicity of cyclophosphamide on the bone marrow. The increase in platelets observed with melatonin in the first week of rats exposed to 10mg cyclophosphamide can be as a result of melatonin being acetylated product of serotonin since previous studies have demonstrated involvement of serotonin in megakaryocytopoiesis. Yang et al., (2008) also hypothesized that therapeutic effects of melatonin may be involved in directly stimulating megakaryocytopoiesis and having anti-apoptric effect in megakaryocytopoiesis via activation of Akt/Erksignaling. Again, the significant increase in platelets count in groups co-treated with the XA and melatonin may be attributed to the observed increase in blood cells occasioned by the antioxidant effects of these treatment substances.

On the other hand, the reductions in the coagulation factors (PT and APTT) in groups treated with the XA and melatonin appeared to be equally related to the increases in the platelet count observed in this study, considering the fact that disorders of the haemostatic mechanisms involve a reduction in platelet number. The findings on the effect of *Xylopiaaethiopica* on coagulation factors (PT and APTT) observed in this study is similar to the report by Nwafor (2013), who observed decreases in the PT and APTT in albino rats treated with methanolic extract of fruits of *Xylopiaaethiopica*. The role of platelets in the processes leading to blood clotting is well established, as increase in the number of circulating platelets correlates with increase in platelets aggregation and hastens the clotting process (Sembulingam and Prema, 2010) and may lead to decrease in both PT and APTT. Platelets, along with the blood clotting factors facilitate the blood clotting process by the formation of platelets plug, blood clots and clot retraction (Guyton and Hall, 2006). Hence any mechanism/agent that reduces platelets count consequently slows down the platelet plug and clotting processes and will increase bleeding time and clotting profile. This may be why *Xylopiaaethiopica* extract is traditionally consumed by women after delivery to arrest bleeding

CONCLUSION

This study provided evidence that *Xylopiaaethiopica* is a valuable medicinal food for combating cyclophosphamide induced systemic toxicity. The ameliorative effects of *Xylopiaaethiopica* may be mediated at least through scavenging reactions of free radicals. Thus, *Xylopiaaethiopica* may provide protective

effects for toxicants capable of inducing oxidative stress. Also It can be seen that despite the high potent immunosuppressive effect of cyclophosphamide on blood cells, melatonin and *Xylopiaaethiopica* have shown to exert their ameliorative effects through their antioxidant and antitumor properties. Therefore, they may be of value in the prevention of diseases arising from the oxidative effects of consumed toxicant substances like cyclophosphamide.

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