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

**Udokwu Euphemia Ifeoma, Okoroiwu I. L., Okolie N. J. C. Anonde Andrew Chekwube and Obeagu Emmanuel Ifeanyi***

Department of Medical Laboratory Science, Imo State University, Owerri, Nigeria.

***Corresponding Author: Obeagu Emmanuel Ifeanyi**

Department of Medical Laboratory Science, Imo State University, Owerri, Nigeria.

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**ABSTRACT**

The study was done to evaluate the effects of Xylopia aethiopica and melatonin on platelets and coagulation profile in cyclophosphamide intoxicated adult wistar rats. Pods of Xylopia aethiopica 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 employed. In week one, and two, there was significant decrease in platelet in all the treatment groups compared with control except week three. Platelet varied significantly from week one to three in all the treatment groups except group 2 and 13. The results showed in week 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 Xylopia aethiopica alone and 400mg Xylopia aethiopica and 0.5mg Melatonin significantly increased the platelet count of rats exposed to 10mg Cyclophosphamide. Platelet count of rats treated with 400 mg Xylopia aethiopica and 0.5mg Melatonin was significantly higher than 400mg Xylopia aethiopica alone. Similarly, treatment with 400mg Xylopia aethiopica alone and 400mg Xylopia aethiopica and 0.5mg Melatonin significantly increased the platelet of rats exposed to 30mg Cyclophosphamide. Platelet count of rats treated with 400mg Xylopia aethiopica and 0.5mg Melatonin was significantly higher than 400mg Xylopia aethiopica alone. Treatment with 400mg Xylopia aethiopica and 0.5mg Melatonin significantly increased the platelet of rats exposed to 50mg Cyclophosphamide. In week three, treatment with 400mg Xylopia aethiopica and 0.5 mg Melatonin alone and in combination did not significantly increase the platelet of rats exposed to 10mg Cyclophosphamide. In week one and two and three, there was significant increase in average PT in all the treatment groups compared with control except group 1 and 3 in week one. Average PT varied significantly from week one to three in all the treatment except group 13. In week 1, average PT of rats exposed to 10 mg Cyclophosphamide and treated with 400 mg Xylopia aethiopica and 0.5 mg Melatonin alone was significantly different from rats exposed to 10 mg Cyclophosphamide.Average PT of rats treated with 400mg Xylopia aethiopica and 0.5mg Melatonin singly and in combination was not significantly different from the average PT of rats exposed to 30mg Cyclophosphamide. Similarly, average PT of rats treated with 400mg Xylopia aethiopica and 0.5 mg Melatonin was not significantly different from the average PT of rats exposed to 50mg Cyclophosphamide. By week 2, average PT of rats exposed to 10mg Cyclophosphamide and treated with 400 mg Xylopia aethiopica alone was significantly different from rats treated with 10 mg Cyclophosphamide alone. Average PT of rats exposed to 30 mg Cyclophosphamide and treated with 400mg Xylopia aethiopica and 0.5mg Melatonin singly and in combination was significantly higher than rats treated with 30mg Cyclophosphamide alone. Similarly, average PT of rats exposed to 50 mg Cyclophosphamide and treated with 400 mg Xylopia aethiopica and0.5mg melatonin singly and in combination was significantly different from rats treated with 50mg Cyclophosphamide alone. In week three, average PT of rats exposed to 10mg Cyclophosphamide treated with 400 mg Xylopia aethiopica and 0.5 mg Melatonin alone and in combination was significantly different from rats treated with 10mg Cyclophosphamide. In week one, two and three, there was significant increase in APTT in all the treatment groups compared with control except group 1 and 3 in week one. APTT varied significantly from week one to three in all the treatment except group 13. In week one and two, there was a dose dependent increase in APTT of rats treated with 10, 30 and 50 mg/kg of Cyclophosphamide. In week 1 and 2, treatment with 400mg Xylopia aethiopica alone, 0.5mg Melatonin alone and 400mg Xylopia aethiopica and 0.5mg Melatonin significantly increased the APTT of rats exposed to 10mg Cyclophosphamide. Treatment with 0.5mg of Melatonin and 0.5mg of Melatonin combined with 400mg Xylopia aethiopica decreases APTT of exposed rats compared with rats treated with 30mg Cyclophosphamide. 400mg Xylopia aethiopica alone significantly increased the APTT of rats exposed to 30mg Cyclophosphamide. APTT of rats treated with 400mg Xylopia aethiopica alone and 0.5mg Melatonin alone was significantly higher compared to expose to 50mgCcyclophophamide. In week three, treatment with 400mg Xylopia aethiopica and 0.5mg Melatonin significantly increased average APTT of rats exposed to 10mg Cyclophosphamide.

**KEYWORDS**: Xylopia aethiopica, melatonin, platelets, coagulation profile, cyclophosphamide intoxicated wistar 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 system have 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 ($G^{\bullet}$) 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.

*Xylopia aethiopica*, a shrub locally referred to as Ethiopian pepper, Negro pepper, Guinean pepper, Senegal pepper, Kili pepper and spice tree in the savanna zone 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. *Xylopia aethiopica* 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).

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 *Xylopia aethiopica* and melatonin on platelets and coagulation profile, in cyclophosphamide induced wistar rats.

## AIM

The study was done to evaluate the effects of *Xylopia aethiopica* and melatonin on platelets and coagulation profile in cyclophosphamide intoxicated adult wistar rats.

## MATERIALS AND METHODS

### Collection of plant materials and authentication

Pods of *Xylopia aethiopica* were purchased from Orie-Ugba 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 sample of 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 aluminum cages 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: 10 mg/kg Cyclophosphamide, 400 mg/kg Extract, Food and water
Group III: 10 mg/kg Cyclophosphamide, 0.5 mg/kg Melatonin, Food and water
Group IV: 10 mg/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, 400 mg/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 haematology and osmotic fragility studies. Liver and kidney samples were also collected and preserved in 10% formalin for histological examination.

**Haematological studies**

Platelets (PLT) counts were determined. An Automated Haematology Analyser (Mindray company, China), was used following the procedures by the producer.

**Determination of activated partial thromboplastin time (APTT) authomated kl340 coagulation analyzer**
**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 100 µl of test plasma was pipette 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 analyzer**
**Procedure**

Prothrombin reagent was dispensed into a thoroughly clean and dry plastic tube for immediate use and pre-warm 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. The mixture was well mixed and incubated at 37$^{0}$C 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 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 employed.

**RESULTS**

**Result of effects of *Xylopia aethiopica*extract and Melatonin on Platelet of Cyclophosphamideintoxicated wistar rats**

In week one, and two, there was significant decrease in platelet in all the treatment groups compared with control except week three. Platelet varied significantly from week one to three in all the treatment groups except group 2 and 13.

In week 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 *Xylopia aethiopica* alone and 400mg *Xylopia aethiopica* and 0.5mg Melatonin significantly increased the platelet count of rats exposed to 10mg Cyclophosphamide. Platelet count of rats treated with 400 mg *Xylopia aethiopica* and 0.5mg Melatonin was significantly higher than 400mg *Xylopia aethiopica* alone. Similarly, treatment with 400mg *Xylopia aethiopica* alone and 400mg *Xylopia aethiopica* and 0.5mg Melatonin significantly increased the platelet of rats exposed to 30mg Cyclophosphamide. Platelet count of rats treated with 400mg *Xylopia aethiopica* and 0.5mg Melatonin was significantly higher than 400mg *Xylopia aethiopica* alone. Treatment with 400mg *Xylopia aethiopica* and 0.5mg Melatonin significantly increased the platelet of rats exposed to 50mg Cyclophosphamide.

In week three, treatment with 400mg *Xylopia aethiopica* and 0.5 mg Melatonin alone and in combination did not significantly increase the platelet of rats exposed to 10mg Cyclophosphamide.

**Table 1: Effects of *Xylopia aethiopica* extract and Melatonin on Platelet of Cyclophosphamideintoxicated wistar rats.**

| Treatment | Platelet | | | (Wk1) (Wk2) (Wk3) | | | A.Wk | |
|---|---|---|---|---|---|---|---|---|
| | Week 1 (Wk1) | Week 2 (Wk2) | Week 3 (Wk3) | p-value | p-value | p-value | p-value | F-value |
| 10mgCyclophosphamide | 37±0.29 $^{k}$ | 32±0.17 $^{k}$ | 25.6±0.11 $^{a}$ | .000 | .000 | .001 | .000 | 20247.621 |
| 10mg Cyclophosphamide+ 400mg *Xylopia aethiopica* | 38.7±0.35 $^{c}$ | 39.1±0.11 $^{d}$ | 35.8±0.06 $^{a}$ | .000 | .000 | .002 | .999 | .001 |
| 10mg Cyclophosphamide + 0.5mg Melatonin | 38.5±0.23 $^{j}$ | 31.9±0.05 $^{h}$ | | .000 | .000 | | .000 | 145.200 |
| 10mg Cyclophosphamide+ 400mg *Xylopia aethiopica* + 0.5mg Melatonin | 40.9±0.06 $^{i}$ | 36.9±0.05 $^{g}$ | 32.7±0.06 $^{a}$ | .000 | .000 | .001 | .000 | 5683.568 |
| 30mg Cyclophosphamide | 35.8±0.06 $^{j}$ | 30.1±0.05 $^{e}$ | | .000 | .000 | | .000 | 3307.500 |
| 30mg Cyclophosphamide+ 400mg *Xylopia aethiopica* | 38.5±0.12 $^{d}$ | 32.2±0.05 $^{bc}$ | | .000 | .000 | | .000 | 672.923 |
| 30mg Cyclophosphamide + 0.5mg Melatonin | 33.6±0.06 $^{g}$ | 28.2±0.05 $^{d}$ | | .000 | .000 | | .000 | 1559.294 |
| 30mg Cyclophosphamide+ 400mg *Xylopia aethiopica* + 0.5mg Melatonin | 38.3±0.12 $^{f}$ | 30.9±0.05 $^{c}$ | | .000 | .000 | | .000 | 4455.375 |
| 50mg Cyclophosphamide | 28.5±0.12 $^{e}$ | 28.2±0.05 $^{e}$ | | .000 | .000 | | .001 | 86.400 |
| 50mg Cyclophosphamide+ 400mg *Xylopia aethiopica* | 32.2±0.06 $^{a}$ | 30.8±0.74 $^{a}$ | | .000 | .000 | | .000 | 240.000 |
| 50mg Cyclophosphamide + 0.5mg Melatonin | 28.5±0.06 $^{c}$ | 26.5±0.06 $^{bc}$ | | .000 | .000 | | .008 | 24.870 |
| 50mg Cyclophosphamide+ 400mg *Xylopia aethiopica* + 0.5mg Melatonin | 30±0.29 $^{b}$ | 26.9±0.06 $^{b}$ | | .000 | .000 | | .000 | 3.594 |
| Control | 45.6±0.06 $^{l}$ | 43.1±0.06 $^{j}$ | 44.3±0.23 $^{b}$ | | | | .131 | 90.067 |

**Result of effects of *Xylopia aethiopica* extract and Melatonin on PT (s) of Cyclophosphamide intoxicated wistar rats**

In week one and two and three, there was significant increase in average PT in all the treatment groups compared with control except group 1 and 3 in week one. Average PT varied significantly from week one to three in all the treatment except group 13.

In week 1, average PT of rats exposed to 10 mg Cyclophosphamide and treated with 400 mg *Xylopia aethiopica and* 0.5 mg Melatonin alone was significantly different from rats exposed to 10 mg Cyclophosphamide. Average PT of rats treated with 400mg *Xylopia aethiopica* and 0.5mg Melatonin singly and in combination was not significantly different from the average PT of rats exposed to 30mg Cyclophosphamide. Similarly, average PT of rats treated with 400mg *Xylopia aethiopica* and 0.5 mg Melatonin was not significantly different from the average PT of rats exposed to 50mg Cyclophosphamide.

By week 2, average PT of rats exposed to 10mg Cyclophosphamide and treated with 400 mg *Xylopia aethiopica* alone was significantly different from rats treated with 10 mg Cyclophosphamide alone. Average PT of rats exposed to 30 mg Cyclophosphamide and treated with 400mg *Xylopia aethiopica and* 0.5mg Melatonin singly and in combination was significantly higher than rats treated with 30mg Cyclophosphamide

alone. Similarly, average PT of rats exposed to 50 mg Cyclophosphamide and treated with 400 mg *Xylopia aethiopica* and0.5mg melatonin singly and in combination was significantly different from rats treated with 50mg Cyclophosphamide alone.

In week three, average PT of rats exposed to 10mg Cyclophosphamide treated with 400 mg *Xylopia aethiopica* and 0.5 mg Melatonin alone and in combination was significantly different from rats treated with 10mg Cyclophosphamide.

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

| Treatment | PT (s) | | | (Wk1) (Wk2) | | (Wk3) A.Wk | | |
|---|---|---|---|---|---|---|---|---|
| | Week 1 (Wk1) | Week 2 (Wk2) | Week 3 (Wk3) | p-value | p-value | p-value | p-value | f-value |
| 10mg Cyclophosphamide | 13.9±0.12 $^{ab}$ | 18.9±0.06 $^{b}$ | 20±0.06 $^{b}$ | .434 | .000 | .000 | .000 | 1585.500 |
| 10mg Cyclophosphamide+ 400mg *Xylopia aethiopica* | 17±0.06 $^{c}$ | 20.1±0.12 $^{de}$ | 21±0.17 $^{c}$ | .000 | .000 | .000 | .000 | 283.071 |
| 10mg Cyclophosphamide + 0.5mg Melatonin | 14.3±0.35 $^{ab}$ | 19.2±0.17 $^{bc}$ | | .059 | .000 | | .000 | 160.067 |
| 10mgCyclophosphamide+ 400mg *Xylopia aethiopica* + 0.5mg Melatonin | 15.1±0.12 $^{b}$ | 19±0.12 $^{b}$ | 20.9±0.12 $^{c}$ | .000 | .000 | .000 | .000 | 655.750 |
| 30mg Cyclophosphamide | 17.4±0.23 $^{cd}$ | 19.7±0.06 $^{cd}$ | | .000 | .000 | | .001 | 93.353 |
| 30mgCyclophosphamide + 400mg *Xylopia aethiopica* | 17.9±0.17$^{cde}$ | 21±0.17 $^{f}$ | | .000 | .000 | | .000 | 160.167 |
| 30mg Cyclophosphamide + 0.5mg Melatonin | 17.1±0.12$^{c}$ | 20.5±0.06 $^{ef}$ | | .000 | .000 | | .000 | 693.600 |
| 30mg Cyclophosphamide+ 400mg *Xylopia aethiopica* + 0.5mg Melatonin | 17.3±0.12 $^{cd}$ | 20.7±0.06 $^{ef}$ | | .000 | .000 | | .000 | 693.600 |
| 50mg Cyclophosphamide | 18.1±0.69 $^{a}$ | 20.8±0.23 $^{f}$ | | .000 | .000 | | .021 | 13.669 |
| 50mg Cyclophosphamide+ 400mg *Xylopia aethiopica* | 18.9±0.12 $^{cde}$ | 21.9±0.12 $^{h}$ | | .000 | .000 | | .000 | 337.500 |
| 50mg Cyclophosphamide + 0.5mg Melatonin | 18.5±0.17 $^{de}$ | 21.1±0.12 $^{fg}$ | | .000 | .000 | | .000 | 156.000 |
| 50mgCyclophosphamide+ 400mg *Xylopia aethiopica* + 0.5mg Melatonin | 18±0.17 $^{cde}$ | 21.7±0.12 $^{gh}$ | | .000 | .000 | | .000 | 315.923 |
| Control | 13±0.23 $^{a}$ | 13.8±0.06 $^{a}$ | 13.7±0.12 $^{a}$ | | | | .090 | 8.143 |

**Result of effects of *Xylopia aethiopica* extract and Melatonin on APTT (s) of Cyclophosphamideintoxicated wistar rats**

In week one, two and three, there was significant increase in APTT in all the treatment groups compared with control except group 1 and 3 in week one. APTT varied significantly from week one to three in all the treatment except group 13.

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

In week 1 and 2, treatment with 400mg *Xylopia aethiopica* alone, 0.5mg Melatonin alone and 400mg *Xylopia aethiopica* and 0.5mg Melatonin significantly increased the APTT of rats exposed to 10mg Cyclophosphamide.

Treatment with 0.5mg of Melatonin and 0.5mg of Melatonin combined with 400mg *Xylopia aethiopica* decreases APTT of exposed rats compared with rats treated with 30mg Cyclophosphamide. 400mg *Xylopia aethiopica* alone significantly increased the APTT of rats exposed to 30mg Cyclophosphamide. APTT of rats treated with 400mg *Xylopia aethiopica* alone and 0.5mg Melatonin alone was significantly higher compared to exposed to 50mgCcyclophophamide.

In week three, treatment with 400mg *Xylopia aethiopica* and 0.5mg Melatonin significantly increased average APTT of rats exposed to 10mg Cyclophosphamide.

**Table 3: Effects of *Xylopia aethiopica* extract and Melatonin on APTT (s) of Cyclophosphamide intoxicated wistar rats.**

| Treatment | AVERAGE APTT (s) | | | (Wk1) | (Wk2) | (Wk3) | A.W k | |
|---|---|---|---|---|---|---|---|---|
| | Week 1 (Wk1) | Week 2 (Wk2) | Week 3 (Wk3) | p-value | p-value | p-value | p-value | F-value |
| 10mg Cyclophosphamide | 30.3±0.06 [a] | 34.3±0.06 [b] | 39.2±0.17 [b] | .489 | .000 | .000 | .000 | 1625.727 |
| 10mg Cyclophosphamide +400mg *Xylopia aethiopica* | 35.3±0.17 [cd] | 35±0.06 [c] | 40.93±0.09 [c] | .000 | .000 | .000 | .000 | 815.216 |
| 10mg Cyclophosphamide + 0.5mg Melatonin | 32±0.58 [b] | 35.9±0.17 [d] | | .000 | .000 | | .003 | 41.862 |
| 10mg Cyclophosphamide+400mg *Xylopia aethiopica* + 0.5mg Melatonin | 34.5±0.12 [cd] | 34.4±0.06 [b] | 39.3±0.06 [b] | .000 | .000 | .000 | .000 | 1176.500 |
| 30mg Cyclophosphamide | 37.5±0.23 [f] | 38.3±0.06 [e] | | .000 | .000 | | .028 | 11.294 |
| 30mg Cyclophosphamide +400mg *Xylopia aethiopica* | 39.1±0.23 [g] | 40.6±0.06 [g] | | .000 | .000 | | .003 | 39.706 |
| 30mg Cyclophosphamide + 0.5mg Melatonin | 34.4±0.12 [c] | 38.4±0.06 [e] | | .000 | .000 | | .000 | 960.000 |
| 30mgCyclophoshamide+400mg *Xylopia aethiopica* + 0.5mg Melatonin | 35.9±0.06 [cde] | 39.1±0.12 [f] | | .000 | .000 | | .000 | 614.400 |
| 50mg Cyclophosphamide | 37±0.06 [ef] | 51.3±0.12 [a] | | .000 | .000 | | .000 | 12269.400 |
| 50mg Cyclophosphamide +400mg *Xylopia aethiopica* | 46.8±0.06 [i] | 51.6±0.17 [hi] | | .000 | .000 | | .000 | 691.200 |
| 50mg Cyclophosphamide + 0.5mg Melatonin | 42.2±0.64 [h] | 51±0.06 [h] | | .000 | .000 | | .000 | 190.426 |
| 50mg Cyclophosphamide +400mg *Xylopia aethiopica* + 0.5mg Melatonin | 36.4±0.35 [def] | 51.4±0.17 [hi] | | .000 | .000 | | .000 | 1500.000 |
| Control | 29.3±0.35 [a] | 29±0.12 [a] | 29.07±0.03 [a] | | | | .602 | .554 |

## DISCUSSION

The fall in platelets counts following CP administration suggests thrombocytopenia may have occurred due to the cytotoxic effects of CP (Friken and Barnes, 1988; Ukpo *et al.*, 2017). Our results also show a significant increase mean platelet rats treated with 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 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/Erk signaling.The fact that the extract was able to lower platelets counts may be why it further increased both prothrombin time (PT) and activated partial thromboplastin time (APTT) in the test animals, suggesting that the extract may have anti-platelets aggregation and possible fibrinolytic activity. Falls in platelets counts have directly being linked to increased bleeding and clotting times and has a little advantage of reducing the risk of blood clots being developed within the blood vessels and its consequent cardiovascular problems (Inyang *et al.*, 2011; Torres-Uruttia *et al.*, 2011; Akomas and Ijioma, 2014).

## CONCLUSION

This study provides evidence that *Xylopia aethiopica* is a valuable medicinal food for combating cyclophosphamide induced systemic toxicity. The ameliorative effects of *Xylopia aethiopica* may be mediated at least through scavenging reactions of free radicals. Thus, *Xylopia aethiopica* 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 *Xylopia aethiopica* have shown to exert their ameliorative effects through their antioxidant and antitumour properties. Therefore, they may be of value in the prevention of diseases arising from the oxidative effects of consumed toxicant substances like cyclophosphamide.

**REFERENCES**


1. Akomas S.C., Okafor A.I., and Ijioma, S.N (2014). Hypoglycemic, Hematologic and Hypolipidemic Activity of *Mucuna Pruriens*ethanol leaf extract in alloxan induced diabetic rats. *Annual Research and Review in Biology*, 4(24): 4284-4292.
2. Azevedo, L., Alves de Lima, P. L., Gomes, J. C., Stringheta, P. C., Ribeiro, D. A., and Salvadori, D. M. (2007). Differential response related to genotoxicity between eggplant (Solanummelanogena) skin aqueous extract and its main purified anthrocyanin (delphinidin) in vivo. *Food Chemistry and Toxicology*, 45: 852 – 858.
3. Bandiola, T.B. (2018). Extraction and Qualitative Phytochemical Screening of Medicinal Plants: A Brief Summary.International Journal of Pharmacy, 8(1): 137-143.
4. Chabner, B. A., Ryand, D. P., Pax-Ares, L., Garcis-Carbonero, and Calaresi, P. (2001).Antineoplastic Agents. In Goodman and Gilman's The Pharmacological Basis of Therapeutics, 10th ed. J.G. Harnam and L.E. Libmirde, eds New York, NY: McGraw Hill, 1389-1459.
5. Ferreira, S.G., Peliciari-Garcia, R.A., Takahashi-Hyodo, S. A., Rodrigues, A.C., Amaral, F.G., Berra, C.M., Bordin, S., Curi, R., and Cipolla-Neto, J.(2013). Effects of melatonin on DNA damage induced by cyclophosphamide in rats.*Brazilian Journal of Medical and Biological Research*, 46(3): 278-286.
6. Fetse, J., Kofie, W., and Adosraku, R., K.(2016). Ethnopharmacological Importance of *Xylopiaaethiopica* (DUNAL) A. RICH (Annonaceae)- A Review. *British Journal of Pharmaceutical Research*, 11(1): 1-21.
7. Frinken, M.D and Barnes, H.J. (1988). Effect of cyclophosphamide on selected haematological parameters of turkey. *Avian Diseases*, 32(4): 812-817.
8. Gitanjah, K., Renu, C., and Mahindra, N. (2017).Effect of cyclophosphamide on liver in albino rats: A comparative dose dependent histomorphological study. *International Journal of Biomedical and Advance Research*, 8(03): 102–107.
9. Inyang, O.I., Okon, U.A., Ekpeyong, U. (2011). Reduction of platelets and lymphocytes counts and elevation of neutrophil counts in rats treated with aqueous leaf extract of *Ocimum gratissimum. African Journal of Biochemical Research*, 5: 22-28.
10. Jensen, W.B. (2007). The Origin of the Soxhlet Extractor.*Journal of ChemicalEducation*, 84(12): 1913-1914.
11. Mohammed, B. J., Adhraa, B. H., and Alas, S. (2017).Modifying effect of vit. E on cyclophosphamide drug – induced, haematological parameters and cytotoxic effect in male albino rats. *European Journal of Research*, 3(3): 77–82.
12. Obodo, B. N., Iweka, F. K., Obhakhan,J. O., Dada, F.L.,Festus, O. O., Onoyovwi, A. O., Maduagwuna, G. N., and Okoye, C. F.(2013). Hepatic potentials of *Xylopia aethiopic*a leaves in adult Wistar rats.*International Journal of Herbs and Pharmacological Research*, 2(3): 36–41.
13. OECD (2001). Guidelines for the Testing of Chemicals/Section 4: Health Effects Tests no, 423: Acute oral Toxicity – Acute Toxic Class method. Organization for Economic Cooperation and Development, Paris.
14. Slominski, A. T., Hardeland, R., Zmijewski, M. A., Slominski, R. M., Reiter, R. J., and Paus, R. (2018). Melatonin: A Cutaneous Perspective on its Production, Metabolism, and Functions. *Journal of Investigative Dermatology*, 138(3): 490-499.
15. Tan, D. X., Hardeland, R., Manchester, L. C., Korkmaz, A., Ma, S., Rosales-Corral, S., and Reiter, R. J. (2012)."Functional roles of melatonin in plants, and perspectives in nutritional and agricultural science". *Journal of Experimental Botany*, 63(2): 577–97.
16. Torres-Urrutia, C., Guzman, L., Schmeda-Hirschman, G., Moore-Carrasco, R., Alavcon, M., Astuillo, L., Gutierrez, M., Cassarco, G., Yuri, J. A., Aranda, E. and Palomo, I. (2011).Anti-platelet, anti-coagulant and fibrinolytic activity in vitro of extracts from selected fruits and vegetables. *Blood coagulation fibrinolysis*,22(3): 197–205.
17. Ukpo, G.E., Ehieneta, T.S., Adegoke, A.Y., Salako, A.O. (2017). Evaluation of the haematological and biochemical effects of averone, a herbal formulationagainst cyclophosphamide-induced immunomodulation in male rats. *International Journal of Pharmaceutical Sciences and Research*, 027.
18. Xiangqun, X., Jinhai, G., Gangjun, L.and Zhongping, C.( 2014). Evaluation of Optical Coherence Tomography for the Measurement of the Effects of Activators and Anticoagulants on the Blood Coagulation *InVitro*. *IEEE transactions on bio-medical engineering*, 60(8): 2100–2106.
19. Yang, M., Zhou, M., Ye, J.Y., Cheung, Y.F., Chan, S., Ha, S.Y.and Chan, G.C.(2008).The Effect and Underlying Mechanism of Melatonin on Platelet Formation and Survival in a Thrombocytopenic Model. *Blood*, 112: 1241.