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EFFECTS OF *XYLOPIA AETHIOPICA* EXTRACT AND MELATONIN ON OSMOTIC FRAGILITY OF CYCLOPHOSPHAMIDE INTOXICATED WISTAR RATS

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ABSTRACT

The research work is aimed at evaluating the effects of *Xylopia aethiopica* and melatonin on osmotic fragility 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. 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, two and three, there was significant increase in osmotic fragility in all the treatment groups compared with control except group 4 in week one and three. Osmotic fragility varied significantly from week one to three in all the treatment except group 13. In week one and two, there is a dose dependent increase in osmotic fragility of rats treated with 10, 30 and 50mg of Cyclophosphamide. Treatment with 400mg *Xylopia aethiopica* alone and 400mg *Xylopia aethiopica* and 0.5mg Melatonin and 0.5mg Melatonin significantly decreased the osmotic fragility compared with rats exposed to 10mg Cyclophosphamide. Similarly, treatment with 400mg *Xylopia aethiopica* alone and 400mg *Xylopia aethiopica* and 0.5mg Melatonin significantly decreased osmotic fragility of rats exposed to 30mg Cyclophosphamide. Osmotic fragility of rats treated with 400mg *Xylopia aethiopica* and 0.5mg Melatonin was significantly lower than 400mg *Xylopia aethiopica* alone. Treatment with 400mg *Xylopia aethiopica* and 0.5mg Melatonin significantly decreased osmotic fragility of rats exposed to 50mg Cyclophosphamide. In week three, treatment with 400mg *Xylopia aethiopica* alone and in combination 0.5 mg Melatonin significantly decreased the osmotic fragility of rats exposed to 10mg Cyclophosphamide. This study provides evidence that *Xylopia aethiopica* is a valuable medicinal food for combating cyclophosphamide induced systemic toxicity.

KEYWORDS: *xylopia aethiopica, melatonin, osmotic fragility, 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 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).

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.

The red blood cell integrity is largely dependent on its ability to maintain its membrane constituents that are mostly polyunsaturated free fatty acid, and if this is compromised will result to erythrocyte fragility which becomes more fragile with consequent destruction by the macrophages. This erythrocyte fragility or red cell osmotic fragility is the ability of red blood cells to

undergo haemolysis when subjected to stress and the absolute extent of haemolysis can be measured (Rodak, 2007). When this happens the membranes of the cells undergo lipid peroxidation leading to oxidative deterioration of polyunsaturated fatty acid accumulation of reactive oxygen species (ROS) which are associated with tissue damage by clearing off the sialic acid from the cells making them more prone to phagocytosis. Also, the osmotic fragility of red cells can occur from the increased phosphorylation of P38 and JNK genes which promotes increased production of ROS (Robin and Steven, 2000). Factors such as cell's size, surface area to volume ratio, membrane composition and integrity can equally influence the osmotic fragility of the cells (Fischbach, *et al.*, 2008). 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 osmotic fragility in cyclophosphamide induced wistar rats, with a view of finding a lasting solution to the life threatening effects reported about this cytotoxic drug.

AIM

The research work is aimed at evaluating the effects of *Xylopia aethiopica* and melatonin on osmotic fragility in cyclophosphamide intoxicated adult wistar rats.

MATERIALS AND METHODS

COLLECTION OF PLANT MATERIALS AND AU THENTICATION

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 65^0C 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^oC 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.

DETERMINATION OF RED BLOOD CELLS OSMOTIC FRAGILITY

The method of Adenkole and Olurenmi, (2014) was adopted. Sodium chloride solution (200 ml) was prepared for each sample in concentrations ranging from 0.1-0.85% at pH 7.4. A set of test tubes, each containing 5mls of sodium chloride solution of concentration ranging from 0.1 to 0.85% were serially arranged in a test tube rack. One set of test tubes was used to analyze each sample. A drop of the freshly collected blood was placed into each of the ten test tubes using a dropper pipette and each was mixed by gently inverting the test tubes about three times. The test tubes were then allowed to stand at room temperature for 30 minutes and then centrifuged at 3000 rpm for 10 minutes before reading the absorbance of the supernatant in each test tube on a spectrophotometer at 540nm. The same procedure was repeated for each sample collected. Percentage haemolysis was calculated using the expression:

$$\text{Percentage Haemolysis} = \frac{\text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

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 osmotic fragility (0) scores of Cyclophosphamide intoxicated wistar rats

In week one and two and three, there was no significant difference in all the treatment groups compared with the control group.

In week one, two and three, treatment with 400mg *Xylopia aethiopica* and 0.5mg Melatonin alone and in combination did not significantly osmotic fragility of rats exposed to 10, 30 and 50mg Cyclophosphamide.

Table 1: Effects of *Xylopia aethiopica* extract and Melatonin on osmotic fragility (0) scores of Cyclophosphamide intoxicated wistar rats.

Treatment	0			(Wk1)	(Wk2)	(Wk3)	A.Wk	
	Week 1 (Wk1)	Week 2 (Wk2)	Week 3 (Wk3)	p-value	p-value	p-value	p-value	F-value
10m Cyclophosphamide	100±0 [a]	100.27±0.32 [a]	100.6±0.31 [a]	1.000	1.000	.922	.318	1.394
10mg Cyclophosphamide+ 400mg *Xylopia aethiopia*	100±0 [a]	100.27±0.32 [a]	100.3±0.35 [a]	1.000	1.000	1.000	.711	.361
10mg Cyclophosphamide + 0.5mg Melatonin	100±0 [a]	100.27±0.32		1.000	1.000		.449	.703
10mg Cyclophosphamide+ 400mg*Xylopia aethiopia* + 0.5mg Melatonin	100±0 [a]	100.27±0.32 [a]	100.3±0.35 [a]	1.000	1.000	1.000	.711	.361
30mg Cyclophosphamide	100±0 [a]	100.27±0.32 [a]		1.000	1.000		.449	.703
30mg Cyclophosphamide+ 400mg *Xylopia aethiopica*	100±0 [a]	100.27±0.32 [a]		1.000	1.000		.449	.703
30mg Cyclophosphamide + 0.5mg Melatonin	100±0 [a]	100.27±0.32 [a]		1.000	1.000		.449	.703
30mg Cyclophosphamide+ 400mg *Xylopia aethiopica*+ 0.5mgMelatonn	100±0 [a]	100.27±0.32 [a]		1.000	1.000		.449	.703
50mg Cyclophosphamide	100±0 [a]	100.27±0.32 [a]		1.000	1.000		.449	.703
50mg Cyclophosphamide + 400mg *Xylopia aethiopica*	100±0 [a]	100.27±0.32 [a]		1.000	1.000		.449	.703
50mg Cyclophoshamide + 0.5mg Melatonin	100±0 [a]	100.27±0.32 [a]		1.000	1.000		.449	.703
50mg Cyclophosphamide + 400mg *Xylopia aethiopia*+ 0.5mg Melatonin	100±0 [a]	100.27±0.32 [a]		1.000	1.000		.449	.703
Control	100±0 [a]	100.27±0.32 [a]	100.3±0.35 [a]				.711	.361

Result of effects of *Xylopia aethiopica* extract and Melatonin on osmotic fragility (0.1) scores of Cyclophosphamide intoxicated wistar rats

In week one, two and three, there was significant increase in osmotic fragility in all the treatment groups compared with control except group 4 in week one and three. Osmotic fragility varied significantly from week one to three in all the treatment except group 13.

In week one and two, there is a dose dependent increase in osmotic fragility of rats treated with 10, 30 and 50mg of Cyclophosphamide. Treatment with 400mg *Xylopia aethiopica* alone and 400mg *Xylopia aethiopica* and 0.5mg Melatonin and 0.5mg Melatonin significantly decreased the osmotic fragility compared with rats exposed to 10mg Cyclophosphamide. Similarly, treatment with 400mg *Xylopia aethiopica* alone and 400mg *Xylopia aethiopica* and 0.5mg Melatonin significantly decreased osmotic fragility of rats exposed to 30mg Cyclophosphamide. Osmotic fragility of rats treated with 400mg *Xylopia aethiopica* and 0.5mg Melatonin was significantly lower than 400mg *Xylopia aethiopica* alone. Treatment with 400mg *Xylopia aethiopica* and 0.5mg Melatonin significantly decreased osmotic fragility of rats exposed to 50mg Cyclophosphamide.

In week three, treatment with 400mg *Xylopia aethiopica* alone and in combination 0.5 mg Melatonin significantly decreased the osmotic fragility of rats exposed to 10mg Cyclophosphamide.

Table 2: Effects of *Xylopia aethiopica* extract and Melatonin on osmotic fragility (0.1) scores of Cyclophosphamide intoxicated wistar rats.

Treatment	0.1			(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	92.2±0.12 ^{h}	92.05±0.29 ^{e}	97.02±0.69 ^{b}	.000	.000	.000	.000	41.529
10mg Cyclophosphamide+ 400mg *Xylopia aethiopica*	90.1±0.23 ^{g}	89.39±0.28 ^{d}	83.95±0.29 ^{a}	.000	.011	.172	.000	154.267
10mg Cyclophosphamide + 0.5mg Melatonin	89.54±0.28 ^{g}	89.14±0.28 ^{d}		.000	.045		.368	1.029
10mg Cyclophosphamide+ 400mg *Xylopia aethiopica* + 0.5mg Melatonin	88.1±0.06 ^{f}	88.39±0.28 ^{cd}	85.41±0.3 ^{a}	.036	.811	1.000	.000	47.286
30mg Cyclophosphamide	98.2±0.06 ^{j}	97.77±0.31 ^{g}		.000	.000		.242	1.887
30mg Cyclophosphamide + 400mg *Xylopia aethiopica*	95.12±0.01 ^{i}	94.97±0.3 ^{f}		.000	.000		.652	.237
30mg Cyclophosphamide + 0.5mg Melatonin	86.2±0.06 ^{d}	85.73±0.27 ^{b}		.000	.004		.168	2.820
30mg Cyclophosphamide + 400mg *Xylopia aethiopica* + 0.5mg Melatonin	85.21±0.01 ^{c}	85.34±0.27 ^{b}		.000	.000		.662	.223
50mg Cyclophosphamide	98.19±0.01 ^{j}	98.28±0.31 ^{g}		.000	.000		.798	.075
50mg Cyclophosphamide + 400mg *Xylopia aethiopica*	95.15±0.01 ^{i}	94.5±0.3 ^{f}		.000	.000		.096	4.683
50mg Cyclophosphamide + 0.5mg Melatonin	83.12±0.01 ^{b}	89.26±0.28 ^{d}		.000	.023		.000	470.987
50mg Cyclophosphamide + 400mg *Xylopia aethiopica*+ 0.5mg Melatonin	82.12±0.02 ^{a}	83.34±0.26 ^{a}		.000	.000		.010	21.376
Control	87.51±0.01 ^{e}	87.64±0.28 ^{c}	85.37±0.3 ^{a}				.091	29.403

Result of effects of *Xylopia aethiopica* extract and Melatonin on osmotic fragility (0.2) scores of Cyclophosphamide intoxicated wistar rats

In week one and two, there is a dose dependent increase in osmotic fragility of rats treated with 10, 30 and 50 mg of Cyclophosphamide. Osmotic fragility of rats exposed to 10mg Cyclophosphamide and treated with 400mg *Xylopia aethiopica* was significantly higher 10mg Cyclophosphamide alone. Similarly, treatment with 400mg *Xylopia aethiopica* alone and 400mg *Xylopia aethiopica* and 0.5mg Melatonin significantly decreased osmotic fragility of rats exposed to 30mg Cyclophosphamide. Treatment with 400mg *Xylopia aethiopica* and 0.5mg Melatonin alone and in combination significantly decreased osmotic fragility of rats exposed to 50mg Cyclophosphamide.

In week three, treatment with 400mg *Xylopia aethiopica* alone and in combination 0.5mg Melatonin significantly decreased the osmotic fragility of rats exposed to 10mg Cyclophosphamide.

Table 3: Effects of *Xylopia aethiopica* extract and Melatonin on osmotic fragility (0.2) scores of Cyclophosphamide intoxicated wistar rats

Treatment	0.2			(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	88.1±0.01 ef	89.54±0.28 g	91.27±0.5 c	.000	.000	.000	.002	22.699
10mg Cyclophosphamide +400mg *Xylopia aethiopica*	89.2±0.64 g	86.53±0.27 f	83.44±0.29 a	.000	.000	1.000	.000	44.219
10mg Cyclophosphamide + 0.5mg Melatonin	88.99±0.28 fg	88.14±0.28 g		.000	.000		.098	4.635
10mg Cyclophosphamide +400mg *Xylopia aethiopica* + 0.5mg Melatonin	89.02±0.14 f	88.36±0.28 g	85.36±0.3 b	.000	.000	.019	.000	61.092
30mg Cyclophosphamide	90.5±0.14 h	91.65±0.29 g		.000	.000		.024	12.523
30mg Cyclophosphamide +400mg *Xylopia aethiopica*	87.15±0.01 e	85.33±0.27 ef		.000	.362		.003	45.415
30mg Cyclophosphamide + 0.5mg Melatonin	84.4±0.09 d	83.32±0.26 cd		.281	.356		.018	15.076
30mg Cyclophosphamide + 400mg *Xylopia aethiopica* + 0.5mg Melatonin	82.2±0.06 c	83.53±0.26 cd		.000	.683		.008	24.037
50mg Cyclophosphamide	92.1±0.01 i	95.4±0.3 i		.000	.000		.000	119.485
50mg Cyclophosphamide + 400mg *Xylopia aethiopica*	83.1±0.01 c	82.52±0.26 bc		.000	.004		.091	4.909
50mg Cyclophosphamide + 0.5mg Melatonin	80.14±0.02 b	81.56±0.26 b		.000	.000		.005	29.927
50mg Cyclophosphamide + 400mg *Xylopia aethiopica* + 0.5mg Melatonin	78.13±0.03 a	78.31±0.25 a		.000	.000		.510	.522
Control	85.2±0.12 a	84.33±0.27 de	83.39±0.29 a				.005	14.440

Result of effects of *Xylopia aethiopica* extract and Melatonin on osmotic fragility (0.3) scores of Cyclophosphamide intoxicated wistar rats

In week one and two, there is a dose dependent increase in osmotic fragility of rats treated with 10, 30 and 50 mg/kg of Cyclophosphamide. Treatment with 400mg *Xylopia aethiopica* alone and 400mg *Xylopia aethiopica* and 0.5 mg Melatonin and 0.5 mg Melatonin significantly decreased the osmotic fragility compared with rats exposed to 10mg Cyclophosphamide. Similarly, treatment with 400mg *Xylopia aethiopica* alone and 400mg *Xylopia aethiopica* and 0.5mg Melatonin significantly decreased osmotic fragility of rats exposed to 30mg Cyclophosphamide. Osmotic fragility of rats treated with 400mg *Xylopia aethiopica* and 0.5mg Melatonin was significantly lower than 400mg *Xylopia aethiopica* alone. Treatment with 400mg *Xylopia aethiopica* and 0.5mg Melatonin significantly decreased osmotic fragility of rats exposed to 50mg Cyclophosphamide.

In week three, treatment with 400mg *Xylopia aethiopica* alone and in combination 0.5mg Melatonin significantly decreased the osmotic fragility of rats exposed to 10mg Cyclophosphamide.

Table 4: Effects of *Xylopia aethiopica* extract and Melatonin on osmotic fragility (0.3) scores of Cyclophosphamide intoxicated wistar rats.

Treatment	0.3			(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	85.23±0.02 g	86.47±0.27 f	89.17±1.46 b	.000	.000	.000	.044	5.516
10mg Cyclophosphamide + 400mg *Xylopia aethiopica*	81.53±0.01 e	82.72±0.26 cd	80.65±0.28 a	.000	1.000	.444	.002	21.806
10mg Cyclophosphamide + 0.5mg Melatonin	80.39±0.25 d	81.6±0.26 c		.000	.036		.029	11.140
10mg Cyclophosphamide + 400mg *Xylopia aethiopica* + 0.5mg Melatonin	79.08±0.57 c	78.3±0.25 b	79.33±0.28 a	.000	.000	.982	.227	1.919
30mg Cyclophosphamide	90.12±0.05 i	91.57±0.29 h		.000	.000		.008	24.148
30mg Cyclophosphamide + 400mg *Xylopia aethiopica*	89.11±0.05 h	88.65±0.28 g		.000	.000		.186	2.542
30mg Cyclophosphamide + 0.5mg Melatonin	83.02±0.01 f	84.95±0.27 e		1.000	.001		.002	51.160
30mg Cyclophosphamide + 400mg Xylopia Aethiopica + 0.5mg Melatonin	75.23±0.02 b	77.54±0.25 b		.000	.000		.001	87.840
50mg Cyclophosphamide	90.13±0.01 i	97.99±0.31 i		.000	.000		.000	640.370
50mg Cyclophosphamide + 400mg *Xylopia aethiopica*	80.15±0.01 d	81.36±0.26 c		.000	.008		.009	21.890
50mg Cyclophosphamide + 0.5mg Melatonin	79.13±0.02 c	77.4±0.25 b		.000	.000		.002	49.378
50mg Cyclophosphamide + 400mg *Xylopia aethiopica*+ 0.5mg Melatonin	70.12±0.06 a	75.35±0.24 a		.000	.000		.000	447.667
Control	83.1±0.01 f	83.02±0.26 d	78.94±0.28 a				.000	116.704

Result of effects of *Xylopia aethiopica* extract and Melatonin on osmotic fragility (0.4) scores of Cyclophosphamideintoxicated wistar rats

In week one and two, there was a dose dependent increase in osmotic fragility of rats treated with 10, 30 and 50 mg/kg of Cyclophosphamide. Osmotic fragility of rats exposed to 10mg Cyclophosphamide and treated with 400mg *Xylopia aethiopica* and 0.5mg Melatonin was significantly lower than 10mg Cyclophosphamide but osmotic fragility of other treatment was significantly higher compared with 10mg Cyclophosphamide. Similarly, treatment with Melatoninalone and 400mg *Xylopia aethiopica* and 0.5mg Melatonin significantly decreased osmotic fragility of rats exposed to 30mg Cyclophosphamide. Treatment with 400mg *Xylopia aethiopica* and 0.5mg Melatonin alone and in combination significantly decreased osmotic fragility of rats exposed to 50mg Cyclophosphamide.

In week three, treatment with 400mg *Xylopia aethiopica* alone and in combination 0.5 mg Melatonin significantly decreased the osmotic fragility of rats exposed to 10mg Cyclophosphamide

Table 5: Effects of *Xylopia aethiopica* extract and Melatonin on osmotic fragility (0.4) scores of Cyclophosphamide intoxicated wistar rats.

Treatment	0.4			(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	72.12±0.58 ^{e}	70.47±0.22 ^{ef}	79.12±0.51 ^{b}	.000	.145	.000	.000	98.344
10mg Cyclophosphamide + 400mg *Xylopia aethiopica*	75.13±0.06 ^{g}	71.23±0.23 ^{f}	66.54±0.23 ^{a}	.000	.001	.252	.000	511.581
10mg Cyclophosphamide + 0.5mg Melatonin	73.29±0.23 ^{f}	67.42±0.21 ^{c}		.000	.000		.000	347.082
10mg Cyclophosphamide + 400mg *Xylopia aethiopica* + 0.5mg Melatonin	69.01±0.05 ^{c}	66.09±0.21 ^{b}	66.11±0.23 ^{a}	.013	.000	.070	.000	84.995
30mg Cyclophosphamide	78.2±0.1 ^{h}	79.61±0.25 ^{j}		.000	.000		.007	26.773
30mg Cyclophosphamide + 400mg *Xylopia aethiopica*	78.12±0.01 ^{h}	76.42±0.24 ^{i}		.000	.000		.002	48.958
30mg Cyclophosphamide + 0.5mg Melatonin	73.02±0.01 ^{ef}	72.41±0.23 ^{g}		.000	.000		.057	6.989
30mg Cyclophosphamide + 400mg *Xylopia aethiopica* + 0.5mg Melatonin	74.92±0.33 ^{g}	73.92±0.23 ^{h}		.000	.000		.071	5.989
50mg Cyclophosphamide	75.16±0.01 ^{g}	74.96±0.24 ^{h}		.000	.000		.448	.707
50mg Cyclophosphamide + 400mg *Xylopia aethiopica*	69.17±0.01 ^{c}	68.36±0.22 ^{cd}		.049	.066		.020	13.889
50mg Cyclophosphamide + 0.5mg Melatonin	60.15±0.02 ^{a}	65.37±0.21 ^{b}		.000	.000		.000	629.949
50mg Cyclophosphamide + 400mg *Xylopia aethiopica* + 0.5mg Melatonin	65.12±0.01 ^{b}	63.29±0.2 ^{a}		.000	.000		.001	83.202
Control	70.21±0.12 ^{d}	69.48±0.22 ^{de}	67.47±0.24 ^{a}				.000	50.546

Result of effects of *Xylopia aethiopica* extract and Melatonin on osmotic fragility (0.5) scores of Cyclophosphamide intoxicated wistar rats

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

Osmotic fragility of rats exposed to 10mg Cyclophosphamide and treated with 400mg *Xylopia aethiopica* and 0.5 mg Melatonin alone and in combination was significantly lower than 10mg Cyclophosphamide. Similarly, treatment with Melatoninalone significantly decreased osmotic fragility of rats exposed to 30mg Cyclophosphamide. Treatment with 400mg *Xylopia aethiopica* and 0.5mg Melatonin alone and in combination significantly decreased osmotic fragility of rats exposed to 50mg Cyclophosphamide.

In week three, osmotic fragility of rats exposed to 10mg Cyclophosphamide and treated with 400mg *Xylopia aethiopica* alone and in combination 0.5 mg Melatonin was not significantly different from of rats exposed to 10mg Cyclophosphamide.

Table 6: Effects of *Xylopia aethiopica* extract and Melatonin on osmotic fragility (0.5) scores of Cyclophosphamide intoxicated wistar rats.

Treatment	0.5			(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	33.21±1.61 [d]	33.3±0.11 [d]	35.73±2.36 [b]	1.000	.000	.000	.513	.747
10mg Cyclophosphamide + 400mg *Xylopia aethiopica*	30.15±0.05 [c]	25.17±0.08 [b]	30.9±0.11 [b]	.376	.000	.008	.421	1.004
10mg Cyclophosphamide + 0.5mg Melatonin	26.58±0.08 [b]	23.74±0.08 [a]		1.000	.105		.000	633.634
10mg Cyclophosphamide + 400mg *Xylopia aethiopica* + 0.5mg Melatonin	31.12±0.05 [cd]	31.2±0.1 [ca]	31.21±0.11 [b]	1.000	.000	.006	.737	.321
30mg Cyclophosphamide	40.13±0.02 [e]	40.24±0.13 [f]		1.000	.000		.456	.681
30mg Cyclophosphamide + 400mg *Xylopia aethiopica*	40.13±0.01 [e]	40.24±0.13 [f]		1.000	.000		.451	.697
30mg Cyclophosphamide + 0.5mg Melatonin	38.12±0.02 [e]	38.22±0.12 [e]		1.000	.000		.453	.689
30mg Cyclophosphamide + 400mg *Xylopia aethiopica* + 0.5mg Melatonin	40.12±0.01 [e]	40.23±0.13 [f]		1.000	.000		.451	.697
50mg Cyclophosphamide	56.16±0.03 [h]	56.31±0.18 [i]		1.000	.000		.454	.685
50mg Cyclophosphamide + 400mg *Xylopia aethiopica*	48.13±0.01 [g]	48.26±0.15 [h]		1.000	.000		.450	.699
50mg Cyclophosphamide + 0.5mg Melatonin	44.15±0.01 [f]	44.27±0.14 [g]		1.000	.000		.449	.702
50mg Cyclophosphamide + 400mg *Xylopia aethiopica* + 0.5mg Melatonin	40.15±0.01 [e]	40.26±0.13 [f]		1.000	.000		.449	.702
Control	23.1±0.58 [a]	23.17±0.07 [a]	23.18±0.08 [a]				.985	.015

Result of effects of *Xylopia aethiopica* extract and Melatonin on osmotic fragility (0.6) scores of Cyclophosphamideintoxicated wistar rats

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

In week one, osmotic fragility of rats exposed to 10mg Cyclophosphamide and treated with 400mg *Xylopia aethiopica* and 0.5mg Melatonin alone and in combination was not significantly different compared to rats exposed to 10mg Cyclophosphamide. Similarly, osmotic fragility of rats exposed to 30mg Cyclophosphamide and treated with 400mg *Xylopia aethiopica* and 0.5mg Melatonin alone and in combination was not significantly different compared to rats exposed to 30 mg Cyclophosphamide. Treatment with 0.5mg Melatonin alone and in combination with 400mg *Xylopia aethiopica*significantly decreased osmotic fragility of rats exposed to 50mg Cyclophosphamide.

In week 2, treatment with 400 mg *Xylopia aethiopica* alone and 400 mg *Xylopia aethiopica* and 0.5 mg Melatonin and 0.5mg Melatonin significantly decreased the osmotic fragility compared with rats exposed to 10mg Cyclophosphamide. Similarly, treatment with 400 mg *Xylopia aethiopica* and 0.5mg Melatonin significantly decreased osmotic fragility of rats exposed to 30mg Cyclophosphamide. Treatment with 400mg *Xylopia aethiopica* and 0.5mg Melatonin significantly decreased osmotic fragility of rats exposed to 50mg Cyclophosphamide.

In week three, osmotic fragility of rats exposed to 10mg Cyclophosphamide and treated with 400mg *Xylopia aethiopica* alone and in combination 0.5 mg Melatonin was significantly lower than rats exposed to 10mg Cyclophosphamide.

Table 7: Effects of *Xylopia aethiopica* extract and Melatonin on osmotic fragility (0.6) scores of Cyclophosphamide intoxicated wistar rats.

Treatment		0.6		(Wk1)	(Wk2)	(Wk3)	A.Wk	
	Week (Wk1)	Week 2 (Wk2)	Week 3 (Wk3)	p-value	p-value	p-value	p-value	f-value
10mg Cyclophosphamide	20.13±0.01 [bc]	20.18±0.06 [d]	20.25±0.06 [d]	.000	.000	.000	.323	1.371
10mg Cyclophosphamide + 400mg *Xylopia aethiopica*	18.13±0.1 [b]	18.18±0.06 [c]	19.62±0.07 [c]	.000	.000	.000	.000	113.968
10mg Cyclophosphamide + 0.5mg Melatonin	17.83±0.06 [b]	18.1±0.06 [c]		.001	.000		.031	10.733
10mg Cyclophosphamide + 400mg *Xylopia aethiopica*+ 0.5mg Melatonin	16.1±0.01 [b]	16.14±0.05 [b]	16.15±0.06 [b]	.007	.000	.000	.712	.359
30mg Cyclophosphamide	20.13±0.02 [bc]	20.18±0.06 [d]		.000	.000		.474	.622
30mg Cyclophosphamide + 400mg *Xylopia aethiopica*	22.15±0.02 [bc]	22.21±0.07 [f]		.000	.000		.470	.635
30mg Cyclophosphamide + 0.5mg Melatonin	20.13±0.02 [bc]	20.18±0.06 [d]		.000	.000		.474	.622
30mg Cyclophosphamide + 400mg *Xylopia aethiopica*+ 0.5mg Melatonin	18.13±0.01 [b]	18.18±0.06 [b]		.000	.000		.457	.676
50mg Cyclophosphamide	36.12±0.02 [e]	36.22±0.11 [i]		.000	.000		.454	.687
50mg Cyclophosphamide+ 400mg *Xylopia aethiopica*a	32.13±0.01 [d]	32.22±0.1 [h]		.000	.000		.452	.694
50mg Cyclophoshamide + 0.5mg Melatonin	26.14±0.06 [cd]	26.21±0.08 [g]		.000	.000		.529	.474
50mg Cyclophosphamide + 400mg *Xylopia aethiopica* + 0.5mg Melatonin	21.13±0.01 [bc]	21.19±0.07 [e]		.000	.000		.455	.683
Control	8.47±4.36 [a]	13.24±0.04 [a]	13.24±0.05 [a]				.365	1.198

Result of effects of *Xylopia aethiopica* extract and Melatonin on osmotic fragility (0.7) scores of Cyclophosphamide intoxicated wistar rats

In week one and two, treatment with 400 mg *Xylopia aethiopica* alone and 400mg *Xylopia aethiopica* and 0.5mg Melatonin and 0.5mg Melatonin significantly decreased the osmotic fragility compared with rats exposed to 10mg Cyclophosphamide. Similarly, treatment with 400 mg *Xylopia aethiopica* and 0.5mg Melatonin significantly decreased osmotic fragility of rats exposed to 30mg Cyclophosphamide. Treatment with 400mg *Xylopia aethiopica* and 0.5mg Melatonin significantly decreased osmotic fragility of rats exposed to 50mg Cyclophosphamide.

In week three, osmotic fragility of rats exposed to 10mg Cyclophosphamide and treated with 400 mg *Xylopia aethiopica* alone and in combination 0.5mg Melatonin was significantly lower than rats exposed to 10mg Cyclophosphamide.

Table 8: Effects of *Xylopia aethiopica* extract and Melatonin on osmotic fragility (0.7) scores of Cyclophosphamideintoxicated wistar rat.

Treatment		0.7		(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	15.19±0.17 [e]	15.24±0.05 [f]	16.28±1 [c]	.000	.000	.000	.395	1.088
10mg Cyclophosphamide + 400mg *Xylopia aethiopica*	12.12±0.05 [c]	12.15±0.04 [c]	13.05±0.05 [b]	.000	.000	.018	.000	133.330
10mg Cyclophosphamide + 0.5mg Melatonin	12.02±0.04 [c]	13.22±0.04 [d]		.000	.000		.000	451.130
10mg Cyclophosphamide + 400mg *Xylopia aethiopica*+ 0.5mg Melatonin	11.13±0.01 [b]	11.16±0.04 [b]	11.16±0.04 [ab]	.000	.000	.582	.722	.345
30mg Cyclophosphamide	18.14±0.03 [g]	18.19±0.06 [h]		.000	.000		.495	.562
30mg Cyclophosphamide + 400mg *Xylopia aethiopica*	20.14±0.01 [h]	20.19±0.06 [i]		.000	.000		.451	.697
30mg Cyclophosphamide + 0.5mg Melatonin	16.14±0.01 [f]	16.18±0.05 [g]		.000	.000		.459	.669
30mg Cyclophosphamide + 400mg *Xylopia aethiopica*+ 0.5mg Melatonin	15.14±0.01 [e]	15.18±0.05 [f]		.000	.000		.452	.693
50mg Cyclophosphamide	21.14±0.02 [i]	21.2±0.07 [j]		.000	.000		.472	.629
50mg Cyclophosphamide + 400mg *Xylopia aethiopica*	15.14±0.01 [e]	15.18±0.05 [f]		.000	.000		.452	.693
50mg Cyclophosphamide + 0.5mg Melatonin	16.14±0.01 [f]	16.18±0.05 [g]		.000	.000		.459	.669
50mg Cyclophosphamide + 400mg *Xylopia aethiopica*+ 0.5mg Melatonin	14.14±0.02 [d]	14.18±0.04 [e]		.000	.000		.497	.556
Control	10.2±0 [a]	10.23±0.03 [a]	10.23±0.04 [a]				.711	.361

Result of effects of *Xylopia aethiopica* extract and Melatonin on osmotic fragility (0.8) scores of Cyclophosphamide intoxicated wistar rats

In week one and two, treatment with 400mg *Xylopia aethiopica* alone and 400mg *Xylopia aethiopica* and 0.5mg Melatonin and 0.5mg Melatonin significantly decreased the osmotic fragility compared with rats exposed to 10mg Cyclophosphamide. Similarly, treatment with 400mg *Xylopia aethiopica* and 0.5mg Melatonin and the combination significantly decreased osmotic fragility of rats exposed to 30mg Cyclophosphamide. Treatment with 400mg *Xylopia aethiopica* and 0.5mg Melatonin significantly decreased osmotic fragility of rats exposed to 50mg Cyclophosphamide.

In week three, osmotic fragility of rats exposed to 10mg Cyclophosphamide and treated with 400mg *Xylopia aethiopica* alone and in combination 0.5mg Melatonin was significantly lower than rats exposed to 10mg Cyclophosphamide.

Table 9: Effects of *Xylopia aethiopica* extract and Melatonin on osmotic fragility (0.8) scores of Cyclophosphamide induced toxicity rats.

Treatment		0.8		(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	13.21±0.76 [d]	13.25±0.04 [e]	13.94±0.66 [c]	.000	.000	.000	.630	.499
10mg Cyclophosphamide+ 400mg *Xylopia aethiopica*	10.14±0.04 [bc]	10.17±0.03 [c]	10.14±0.04 [b]	.000	.000	.005	.837	.183
10mg Cyclophosphamide + 0.5mg Melatonin	9.93±0.03 [b]	9.15±0.03 [b]		.000	.000		.000	332.650
10mg Cyclophosphamide + 400mg *Xylopia aethiopica* + 0.5mg Melatonin	9.04±0.02 [b]	9.06±0.03 [b]	9.07±0.03 [ab]	.021	.000	.109	.744	.311
30mg Cyclophosphamide	15.13±0.1 [e]	15.17±0.05 [f]		.000	.000		.731	.136
30mg Cyclophosphamide+ 400mg *Xylopia aethiopica*	13.13±0 [d]	13.17±0.04 [e]		.000	.000		.449	.703
30mg Cyclophosphamide + 0.5mg Melatonin	11.15±0.02 [c]	11.18±0.04 [d]		.000	.000		.521	.493
30mg Cyclophosphamide + 400mg *Xylopia aethiopica* + 0.5mg Melatonin	11.14±0.01 [c]	11.17±0.04 [d]		.000	.000		.454	.685
50mg Cyclophosphamide	20.13±0.01 [f]	20.18±0.06 [g]		.000	.000		.456	.681
50mg Cyclophosphamide+ 400mg *Xylopia aethiopica*	10.1±0.05 [bc]	10.13±0.03 [c]		.000	.000		.633	.266
50mg Cyclophosphamide + 0.5mg Melatonin	9.12±0.01 [b]	9.14±0.03 [b]		.011	.000		.480	.607
50mg Cyclophosphamide+ 400mg Xylopia Aethiopica + 0.5mg Melatonin	10.12±0.02 [bc]	10.15±0.03 [c]		.000	.000		.501	.545
Control	7.8±0.17 [a]	7.83±0.02 [a]	7.83±0.03 [a]				.973	.027

Result of effects of *Xylopia aethiopica* extract and Melatonin on osmotic fragility (0.85) scores of Cyclophosphamide intoxicated wistar rats

In week one, osmotic fragility of rats at exposed to 10mg Cyclophosphamide and treated with 400mg *Xylopia aethiopica* and 0.5mg Melatonin and the combination was significantly higher than rats treated with 10mg Cyclophosphamide. Treatment with 400mg *Xylopia aethiopica* and 0.5mg Melatonin and the combination significantly decreased osmotic fragility of rats exposed to 30mg Cyclophosphamide. Treatment with 400mg *Xylopia aethiopica* and 0.5mg Melatonin significantly decreased osmotic fragility of rats exposed to 50mg Cyclophosphamide.

In week two, treatment with 400mg *Xylopia aethiopica* alone and 400 mg *Xylopia aethiopica* and 0.5mg Melatonin and 0.5mg Melatonin significantly decreased the osmotic fragility compared with rats exposed to 10mg Cyclophosphamide. Similarly, treatment with 400mg *Xylopia aethiopica* and 0.5 mg Melatonin and the combination significantly decreased osmotic fragility of rats exposed to 30mg Cyclophosphamide. Treatment with 400mg *Xylopia aethiopica* and 0.5mg Melatonin significantly decreased osmotic fragility of rats exposed to 50mg Cyclophosphamide.

In week three, osmotic fragility of rats exposed to 10mg Cyclophosphamide and treated with 400 mg *Xylopia aethiopica* alone and in combination 0.5mg Melatonin was significantly lower than rats exposed to 10mg Cyclophosphamide.

Table 10: Effects of *Xylopia aethiopica* extract and melatonin on osmotic fragility (0.85) scores of cyclophosphamide intoxicated wistar rats

Treatment	0.85			(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	5.7±0.58 ^{abc}	10.74±0.03 ^{h}	11.88±0.12 ^{d}	.750	.000	.000	.000	93.078
10mg Cyclophosphamide + 400mg *Xylopia aethiopica*	6.8±0.18 ^{def}	6.53±0.02 ^{d}	5.72±0.02 ^{c}	.506	.000	.006	.001	27.192
10mg Cyclophosphamide + 0.5mg Melatonin	7.05±0.02 ^{ef}	5.92±0.02 ^{b}		.095	.000		.000	1509.052
10mg Cyclophosphamide + 400mg *Xylopia aethiopica*+ 0.5mg Melatonin	5.3±0.14 ^{abc}	5.22±0.02 ^{a}	4.72±0.02 ^{a}	.064	.000	.001	.006	13.917
30mg Cyclophosphamide	10.01±0.05 ^{h}	12.04±0.04 ^{i}		.000	.000		.000	1150.542
30mg Cyclophosphamide + 400mg *Xylopia aethiopica*	8±0.17 ^{g}	7.03±0.02 ^{e}		.000	.000		.005	31.130
30mg Cyclophosphamide + 0.5mg Melatonin	6.01±0.01 ^{bcd}	8.03±0.03 ^{g}		1.000	.000		.000	6002.250
30mg Cyclophosphamide + 400mg *Xylopia aethiopica* + 0.5mg Melatonin	5.13±0.01 ^{ab}	7.15±0.02 ^{e}		.014	.000		.000	6307.375
50mg Cyclophosphamide	13.12±0.01 ^{i}	17.24±0.05 ^{j}		.000	.000		.000	5618.664
50mg Cyclophosphamide + 400mg *Xylopia aethiopica*	7.12±0.01 ^{efg}	7.74±0.02 ^{f}		.054	.000		.000	608.567
50mg Cyclophosphamide + 0.5mg Melatonin	7.13±0.01 ^{fg}	5.14±0.02 ^{a}		.050	.000		.000	9891.754
50mg Cyclophosphamide + 400mg *Xylopia aethiopica*+ 0.5mg Melatonin	5.01±0.01 ^{a}	5.91±0.02 ^{b}		.004	.000		.000	1659.689
Control	6.2±0.06 ^{cde}	6.22±0.02 ^{c}	5.31±0.02 ^{b}				.000	200.305

DISCUSSION

Results of osmotic fragility scores indicates that CP significantly reduced RBC percentage haemolysis in the treated rats when compared with the control in different salt concentrations. The result suggest that XA may have increased the integrity of the RBC cell membranes following treatment and made them resist the haemolytic effects of the various salt concentrations. It is established that the performance of normal functions by the erythrocytes is highly dependent on their membrane stability and ability to resist lysis (Adenkola and Oluremi 2014). The impact of free radicals on erythrocyte membrane is a major cause of reduction in its ability to resist lysis (Devasagayam *et al.*, 2004; Dragan *et al.*, 2003). The higher membrane stability observed in all groups treated with XA may be attributed to the antioxidant potentials of the extract. Antioxidants have greatly been implicated in the prevention of cellular damage and generally consolidate the integrity of erythrocyte membrane by reducing their oxidative damage due the impact of free radicals (Adenkola and Oluremi 2014).

CONCLUSION

This study provides evidence that *Xylopia aethiopica* is a valuable medicinal food for combating cyclophosphamide induced systemic toxicity. *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.

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