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Histomorphological Effect of Methanolic Extract of Baobab (*Adansonia digitata*) Fruit on Led Light Induced Retinal Damage in Young Wistar Rats

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ABSTRACT

Changes in modern life style have been implicated to cause retinal damage due to prolonged artificial light exposure. The study investigated the effects of the methanolic extract of *Adansonia digitata* on the histomorphology of the retina in Light Emitting Diode (LED) light induced retinal damage in 16 young wistar rat. The Wistar rats were randomly grouped into 4 groups of 4 animals each. Group A; normal control group, 1ml of normal saline was administered. Group B; negative control, were exposed to LED light. Group C; prevention group, 1300mg/kg of *Adansonia digitata* methanolic extract was administered via 1ml of normal saline for 1 week, followed by exposure to LED light of the intensity (2740 ± 140) lux for 8hr. Group D; therapeutic group, were exposed to LED light of the intensity (2740 ± 140) lux for 8hr, and then followed by the administration of 1300 mg/kg of ADME via 1ml of normal saline for 1 week. All animals were sacrificed on day 15. Part of the results showed that the histology of the retinas in the animals in group B were shrink as seen in the outer nuclear layer, retinal pigmented epithelium and the rod and cone layer. The rats in group C and D showed that ADME preserved partly the outer nuclear layer and rod and cone layer. The study concluded that *Adansonia digitata* methanolic extract possess antioxidant and healing effects capable of protecting and treating partly the retina from LED light induced retinal light damage.

Keywords: histomorphology of the retina, retinal damage, LED Light, Adansonia digitata extract, antioxidant, oxidation.

INTRODUCTION

Retina is the innermost tunic (layer) and the photosensitive layer of the eye[1]. In any sagittal section of the eye ball, the retina occupies the posterior-lateral inner aspect of the eyeball, posterior to the oraserrata[1]. It is divided into outer pigmented and inner retina proper with ten (10) histological layers[2].

In the 21st century, light emitting diode (LED) light is becoming the chief source of artificial light[3]. The light and dark cycle (L/D cycle) of modern humans have remarkably changed over time, people now tend to spend more time of a day in a lighted environment mostly artificial light[3].This changes in modern life style and alteration of the light and dark cycle has caused detrimental effect on the eye, especially the retina[4]. Artificial light such as LED light and fluorescent light has more damaging effects on the retina[5].

Baobab tree is known as "kuka" in Hausa language. Its leaves, pulp and seed are used as food in many parts of Africa[6]. Baobab fruit has been used medically for the treatment of measles, chicken pox, and liver disease and as an eye instillation (eye drop)[7]. It is rich in calcium, vitamins A and C[7]. The fruit pulp contains almost ten times of the vitamin C content of oranges while the leaves contain almost six times that of oranges [8]. Vitamin C has been shown to enhanced immunity against many tropical diseases and lowers the incidence of cataract development[7].

It is a fact that LED light exposure has become inevitable due to the changes in modern life style has been implicated to cause retinal damage. However there is paucity of literature evaluating the preventive and therapeutic effect of baobab fruit on LED light induced retinal tissue damage, despite the fact that this fruit have been proved to have high content of vitamin C and anti-oxidative capabilities than oranges and most other fruits.

This research may provide information that may be important in further studies related to the application of the Baobab fruit in producing a standardized retinal herbal medication.

The research may bring about an available, affordable and edible remedy and prevention of LED light retinal damage.

The aim of the research was to determine the preventive and therapeutic effect of methanolic extract of baobab (*adansonia digitata*) fruit on the histomorphology of the LED light induced retinal damage in young Wistar rats

MATERIALS AND METHODS

Sixteen (16) Wistar rats were procured from the Department of Biological Sciences, Bayero University, Kano. The animals were transported to and housed at the Animal House of the Department of Human Anatomy, Faculty of Basic Medical Science, Yusuf Maitama Sule University, Kano. The Wistar rats were allowed to acclimatize for 14 days. They were then grouped into four (4) groups A, B, C and D contained four (4) wistar rats in each. After 14 days of acclimatization, the study was conducted for a period of 2 weeks, where 1ml of normal saline was used to deliver the *Adansonia digitata*methanolic extract (ADME) to the wistar rats as modified from Xie *et* al.[11]. The 4 groups were treated as follows;

Group A - served as normal control group, not expose to high intensity LED light and no administration of the *Adansonia digitata*methanolic extract (ADME).

Group B – is the experimental control group. The animals in this group

were exposed to high intensity LED (2740 ± 140 lux) light for 6 hours starting from 8am and ending at 2pm on the day 8th of the experiment. But they were not administered ADME. Then the animals were sacrificed after 7 days of exposure (on day 15).

Group C - served as prevention group, where 1300mg/kg of body weight of ADME was administered orally to these animals daily for 1 week. They were then exposed to high intensity LED (2740 ± 140 lux) light for 6 hours starting from 8am and ending at 2pm on the 8th day. The animals were also sacrificed after 7 days of exposure (on day 15).

Group D - served as therapeutic group with 4 animals. The animals in this group were first exposed to high intensity LED light for 6 hours starting from 8am and ending at 2pm on the 8th day of the experiment. 1300mg/kg of body weight of ADME was then administered orally to the animals in this group daily for 1 week. The animals were then sacrificed after 7 days of exposure (on day 15).

LED light system and exposure: The light source used for this research was LED light. The LED lighting system was constructed locally by a trained electrician at Abubakar Rimi Market, Sabon-Gari, Kano State. The lighting system was a rechargeable LED light. The lighting system was constructed specifically for the purpose of the research so as to deliver high illumination and last long hours without the need for recharging. The lighting system was made of white LED light for the purpose of the research.

A light source with an approximate luminance of about 2740 ± 140 lux was used. The light source was suspended from the opaque casing at a height of 30cm above the floor of the cage. The light exposure lasted for 6 hours as adopted from Xie[11]. One (1) Lux (lx) was measured as lumen per square meters; lumen is derived from the watt power of the LED light using an online watt to lumen converter. The lumen is also indicated on the LED light bulb.

An opaque casing was constructed locally using wood in order to house the cages that were used for the research. The opaque casing was constructed to maintain the high intensity light within the cages.

Plant procurement identification and extraction: Pre-dried Baobab fruit was procured from the outskirts of Rano town of Rano LG, Kano state in Nigeria. The fruit was then taken to the Department of Biology, Bayero University, Kano for identification and voucher number issuance.

The pre-dried fruit's shell was cracked in the Histology Laboratory of the Department of Human Anatomy Yusuf Maitama Sule University Kano, to obtain the fruit pulp. The pulp was then obtained from the fruit after cracking the hard dried shell and separating the pulp from the seed using a clean mortar and pestle. The powdered pulp was then stored in a sealed plastic container.

100g of the powdered fruit pulp was measured and extracted using the soxhlet method with 300ml of methanol, until a clear fluid was seen in the siphon tube. The 100g was divided into four batches of 25g each. The solvent in the filtrate was then allowed to evaporate by raising the temperature in a water bath to the boiling point of methanol (65ºC). The resultant sticky methanolic extract was stored at a cool temperature. The extraction process was carried out the Molecular Biology Lab, Faculty of Basic Medical Science, Yusuf Maitama Sule University, Kano.

The LD50 of the methanolic extract of *Adansonia digitata* was between 5000mg/kg [12] and 8000mg/kg[13]. 20% of the average of the LD50 was used in this study as it has been established that it is not toxic[12].

The animals were sacrificed after they were anaesthetized using chloroform at the end of

the experiment, on the 15th day. The animal's heads were immediately severed and the eyeball enucleated, morphological studies were carried out and then immediately fixed in 10% formalin.

Tissue processing for light microscopy was carried out for the morphological and histological appearance of the retina. Routine hematoxylin and eosin was used for light microscopy. Tissue processing was conducted according to steps described by Suvarna *et al*., (2019) [14].

Gross morphological observations of the eyeball such as the shape color and texture of the eyeball for each group was made before fixation. The anterior posterior (AP) diameter of the eyeball of each rat was measured using a vernier caliper before fixation. The weight of each animal was also measured.

The histomorphology of the retinal tissue was observed using the histological slide and a light microscope by visual observation of the histological slides under a light a microscope. The observations were of the distinctness of each of the 10 histological layers of the retina.

The data obtained eyeball anterior posterior diameter and animal weight were presented as mean ± SEM. One way analysis of variance (one way ANOVA) was used to compare between the variables of each group (groups A, B, C and D). A *p*-value of ≤ 0.05 was considered to be statistically significant. SPSS version 20.0 was used.

RESULTS

Morphometric Studies: The morphometric studies of animal weight and anterior posterior eye diameter were conducted and the results were presented as mean ± SEM. The morphometric parameter recorded includes animal weight and the anterior posterior eye diameter. Group A has the largest average animal weight with a value of (126.50 ±7.19) g, group B follows with (115.75±7.71) g, Group C average weight stands as (114.00±11.53) g. Group D has the least average weight (113.75±7.89) g (table 4.1).

Group A animals have the largest anterior posterior eye diameter in all the groups (5.88±0.22) mm, group C animals have the second largest anterior posterior eye diameter in all the groups (5.74±0.24) mm. Group D has an anterior posterior eye diameter of (5.48±0.03) mm as the third largest group in terms of anterior posterior eye diameter. Group B with an AP diameter of (5.45±0.12) mm is the group with the smallest eye diameter (table 4.1).

The result of the one way analysis of variance (ANOVA) shows that the difference between the four groups is not statistically significant in terms of animal weight, first anterior posterior eye diameter, second anterior posterior eye diameter and average anterior posterior eye diameter with a p-value of 0.703, 0.253, 0.248 and 0.249 respectively (table 4.2).

Tukey HSD ANOVA using the multiple comparisons between any 2 groups shows that the difference between every two (2) groups is not statistically significant ($p > 0.05$). Comparison was between group A and B, A and C, A and D, C and B, C and D, and between D and B, the p-value was 0.300, 0.297, 0.367, 0.617, 0.704, 0.999 respectively (table 4.3).

Table 1: Morphometric mean values (± SEM) of the eyeball of the Wistar rat between groups A, B, C and D

VARIABLES	GROUP A (Mean ± SEM)	GROUP B (Mean ± SEM)	GROUP C (Mean ± SEM)	GROUP D (Mean ± SEM)
AW (g)	126.50±7.19	115.75±7.71	114.00±11.53	113.75±7.89
1st APD (mm)	5.89±0.22	5.45± 0.06	5.75±0.25	5.49±0.02
2nd APD (mm)	5.87±0.22	5.45±0.07	5.74±0.24	5.48±0.03
AAPD (mm)	5.88±0.22	5.45±0.06	5.74±0.24	5.48±0.03

AW = animal weight

1st APD = first anterior posterior diameter

2nd APD = second anterior posterior diameter

AAPD = average anterior posterior diameter

Values are expressed as mean ± SEM of the data collected

Table 2: One-way analysis of variance (ANOVA) Morphometric parameters of groups A, B, C and D

	Sum of Squares	Df	Mean Square	F	Sig.
AW (g)	441.500	3	147.167	0.481	0.702
1st APD (mm)	0.519	3	0.173	1.550	0.253
2nd APD (mm)	0.506	3	0.169	1.570	0.248
AAPD (mm)	0.512	3	0.171	1.565	0.249

One-way analysis of variance (ANOVA) of AW, 1st APD, 2nd APD and AAPD

AW = animal weight

1st APD = first anterior posterior diameter

2nd APD = second anterior posterior diameter

AAPD = average anterior posterior diameter

Table 3: Comparisons for the anterior posterior eye diameter (tukey HSD) between each 2 groups

(I) GROUP	(J) GROUP	Mean Difference (I-J)	Sig.
A	B	0.4312500 ± 0.2335650	0.300
A	C	0.1425000 ± 0.2335650	0.927
A	D	0.3962500 ± 0.2335650	0.367
C	B	0.2887500 ± 0.2335650	0.617
C	D	0.2537500 ± 0.2335650	0.704
D	B	0.0350000 ± 0.2335650	0.999

Analysis by tukey HSD one-way analysis of variance (ANOVA) of Wistar ratsAAPD between the groups A and B, A and C, A and D, C and B, C and D, and D and B

AAPD = average anterior posterior diameter

Histology Results: Histological slide prepared using H&E stains for light microscopy revealed that the Wistar rat retina in group A contains 8 histological layers that are typical of a normal histology of the retina; nerve fiber layer (NFL), ganglion cell layer (GL), internal plexiform layer (IPL), retinal pigmented epithelium layer (RPE), inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL), rod and cone layer (R&C). The two membranes; inner limiting membrane between the nerve fiber layer and the vitreous body, the outer limiting membrane between the outer nuclear layer and the rod and cones were barely visible (Plate IV).

Histological studies from the retina of animals in group B showed that the retinal maintained it layered arrangement. However, the layers of the retina are narrowed. Some layers such as the ganglion layer, the nerve fiber layer appear to be scanty and retinal pigmented epithelium layer absent. The outer plexiform layer and the rod and cone layer are also very narrow and scanty with spaces between cells. (Plate VI).

The layers of the retina in group C animals appear to be less in thickness as compared to that of that of those in groups A. however the retinal layers are visible clearly with the exception of the nerve fiber layer and the ganglion cell layer which is almost lost in some regions(Plate VI).

In group D the histological slide has revealed the layers of the retina to also be intact. The retina is however shrunken in thickness. The nerve fiber layer, the ganglion cell layer and are hardly visible in some regions of the retina (Plate VII).

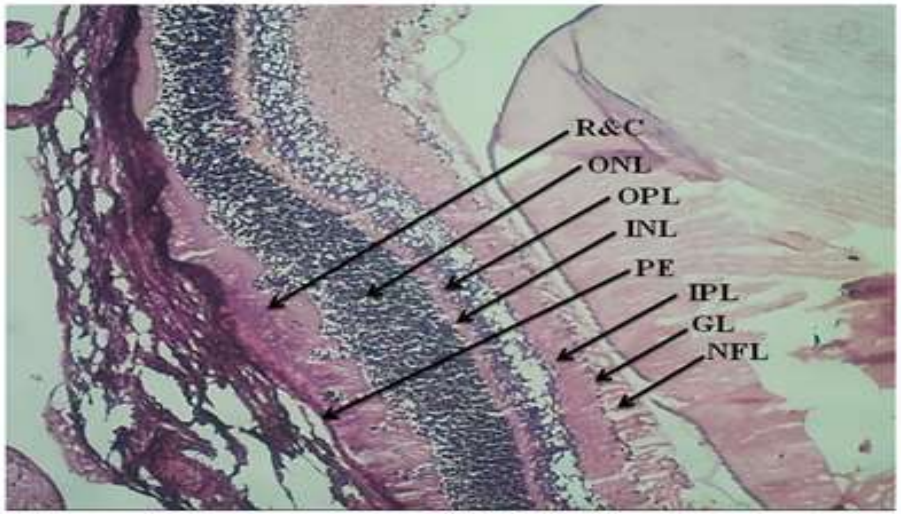

Plate IV: Photomicrograph of the Wistar rat eye in group A showing the nerve fiber layer (**NFL**), ganglion cell layer (**GL**), internal plexiform layer (**IPL**), retinal pigmented epithelium layer (**RPE**), inner nuclear layer (**INL**), outer plexiform layer (**OPL**), outer nuclear layer (**ONL**) and rod and cone layer (**R&C**) x40 H&E

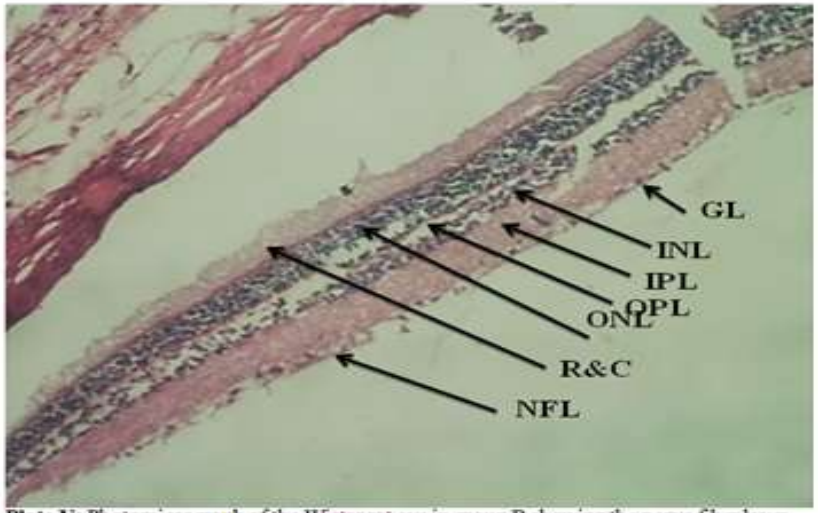

Plate V: Photomicrograph of the Wistar rat eye in group B showing the nerve fiber layer (**NFL**), ganglion cell layer (**GL**), internal plexiform layer (**IPL**), inner nuclear layer (**INL**), outer plexiform layer (**OPL**), outer nuclear layer (**ONL**) and rod and cone layer (**R&C**) x40 H&E

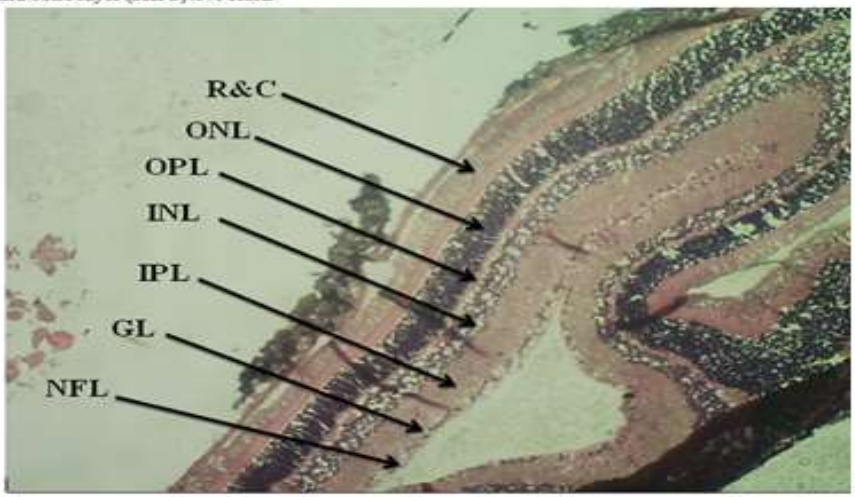

Plate VI: Photomicrograph of the Wistar rat eye in group C showing the nerve fiber layer (**NFL**), ganglion cell layer (**GL**), internal plexiform layer (**IPL**), inner nuclear layer (**INL**), outer plexiform layer (**OPL**), outer nuclear layer (**ONL**) and rod and cone layer (**R&C**) x40 H&E

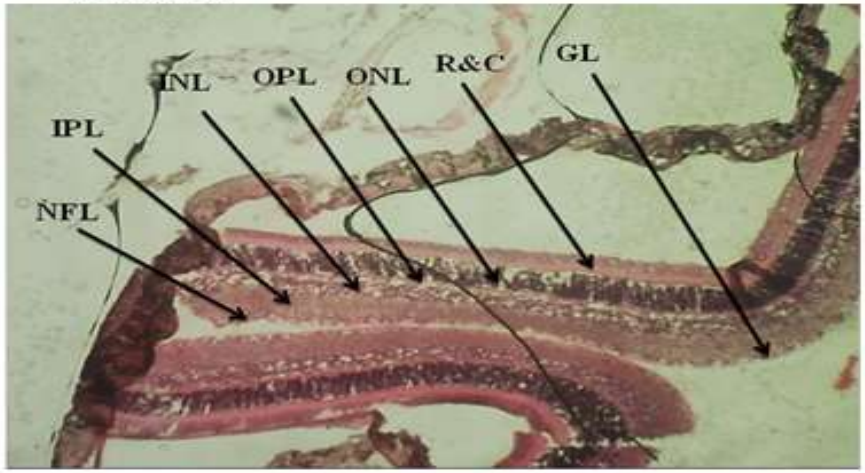

Plate VII: Histomicrograph of the Wistar rat eye in group D showing the nerve fiber layer (**NFL**), ganglion cell layer (**GL**), internal plexiform layer (**IPL**), inner nuclear layer (**INL**), outer plexiform layer (**OPL**), outer nuclear layer (**ONL**) and rod and cone layer (**R&C**) x40 H&E

DISCUSSION

The Wistar rats' eyes were seen to be globular in shape with the optic nerve having an oblique posterior medial direction[15]. The eye in a living Wistar rat appears reddish and glowing when flashed with a light source. This redness of the eyes is due to blood vessels coursing through the retina and choroid, since the animals (Wistar rat) are albino rat, the retinal pigmented epithelium (RPE) and the choroid are transparent[16, 17]. The reflective glowing eye is due to the tapedumlucidum[18] a reflective surface located at the posterior aspect of the retina. This structure which can be found in numerous animals such as dogs, cats, etc. It gives the animals' greater vision during the night [18]. Tendinous like attachments around the corneo-scleral junction are the point of insertions of extra-ocular muscles. The globe has 6 extra-ocular muscles inferior, superior, medial and lateral recti and the inferior and superior oblique[19].

The present studies revealed that the Wistar rat eyes contain two fluid filled chambers; the anterior and posterior chambers containing the aqueous and vitreous fluids respectively [15]. The fluid in the posterior chamber; the vitreous was a sticky substance, while the aqueous is a more fluid like substance. The lens was a transparent biconcave crystalline structure that forms the separation between the two eye chambers. The lens occupies two third of the eye cavity with the posterior curvature larger than the anterior curvature. This feature allows the lens to have a larger diopteric power since the eye diameter is small[15].

The morphologic and morphometric studies show that light exposure does not have an effect on the eye. The anterior posterior diameter of the eyes in each of the four groups does not show any significant difference. Groups A, B, C and D have the following mean diameter 5.88, 5.45, 5.74 and 5.48 respectively which is similar to values reported by Dunn[15], Calkins[20], Shibuya[17], Leopold and Calkins[21]. Morphometric studies have also showed the existence of a positive correlation between animal weight and anterior posterior diameter of the Wistar rat eyes. Song *et al.*[22] has demonstrated such positive correlation between the axial length in human children and their weight and age, similarly, Wisard *et al*.[23] has shown the existence of a positive correlation between axial length and animal weight in mouse.

Results from morphological studies imply that the eye ball size is not grossly affected by the high intensity light in its shape, color, and texture between the normal group (group A) and the exposure untreated group (group B) and other group of the experiment (group C and D). Morphometric studies also do not show any statistically significant differences exist between the normal group (group A) and the exposure group.

Phytochemicals of importance has been found to be present in medicinal plants such as baobab[10]. For thousands of years, plants have the source of medication for human and animals alike. This phenomenon has continued into the present age, and there is a resurgent in the use of medicinal plants as the source of remedy even in developed countries[8]. Baobab, *A. digitata*, has been used for various purposes due to its nutritional and medicinal values; as source of food, medication, fibre for rope making, wood for construction[24]. Of importance is its medicinal properties in which it possess anti-oxidative properties and healing effects caused by the abundance of vitamin C, alkaloids, terpenes, flavonoids, etc., and its anti-inflammatory activities[9, 25]. Preparations from baobab fruit have been used for the treatment of cataract and as eye instillations for other eye ailments[8].

Histological studies has shown that the retina of animals in group A has eight (8) histological layers; retinal pigmented

epithelium (having cuboidal cells), rod and cone layer (processes of the rod and cone cells), outer nuclear layer containing the rod and cone cell body, outer plexiform layer containing the horizontal cells and the synapsis between the bipolar neurons and the rod and cone cells, inner nuclear layer containing the bipolar cell body, inner plexiform layer containing the amacrine cells and the synapsis of the bipolar cells and the ganglion cells, ganglion cell layer composed of the ganglion cells and layer of nerve fiber formed from the axons of the ganglion cells, that are very distinct from each other. This is the normal appearance of the retina conforms to the description of Mescher[2] and Singh[1]. The retinas of Wistar appear lighter in color due to the lack of pigmentation in the retina or the choroid since the animal is a pigmented animal[15].

The retina of animals in group B (exposed untreated) studied using H&E section under a light microscope has demonstrated the layers of the retina. However, the retina appears shrunken in its thickness. The retina has scanty layers, the rod and cone layers (inner and outer) segments are barely separable. Two of the layers containing cell bodies of neural cell (rod and cone cells and the bipolar neurons) degenerated extensively from the effect of LED light exposure. The processes of these cells were also affected by the oxidative effects this is in harmony with the report by Xie *et al*.[11] that states that extensive damage occurs in the outer nuclear layer of the retina (ONL).

Light exposure damage on the retina has been showed to be cause by the excessive absorption of light particles by the pigment molecules of the retina (rhodopsin)[11]. The intensity of light damage to the retina has been showed to be related to the intensity, time and duration of exposure to the light source[11]. The hypothesis is that light exposure during the night time causes more severe damage to the retina than exposure during the day time[11]. Several studies conducted have demonstrated retinal light damage to occur at about 2700 lux including the work of Ranchon *et al.* [23] and Tomita *et al*.[25].

The present experiment found out that *Adansonia digitata* possesses some preventive and therapeutic effects on light induced retinal damage as seen from the histological studies as seen in group C (prevention) and group D (therapy). The ONL as seen from the sample in group B (exposed untreated) appeared thin, scanty and with wide spaces between cells. These spaces are related to the death of cells previously located in that layer. The ONL contains rod and cone cells which contains rhodopsin a molecule that has been implicated in the oxidative damage. As such the extensive damage to the ONL and the outer plexiform layer (OPL) these is similar to finding by Ranchon[23], Tomita[25], [11] and Rozanowka *et al.* (2009)[26] have all attributed the cell death to apoptosis of caused by oxidation in the tissue as was seen in the present study. This in contrast to the ONL of the prevention and therapy group; group B and group C respectively which were able to preserve some of the ONL cells from death or degeneration. The degree of effectiveness of the methanolic extract of the fruit pulp of *Adansonia digitata* on the retina is more prominent in group C (prevention). The ONL of group C from the histological slides appears to maintain roughly the density of cell a seen in the group A (normal). The size or thickness of the layer in both group C (prevention) and group D (therapy) has decreased almost by half.

This protective and therapeutic effect can be attributed mostly to the properties of baobab fruit pulp which includes healing effects, high vitamin C content and antioxidant effects[27, 9]. Other factors such as it rich minerals may also have a role[8].

The PE of the normal group (group A) was evident however, the pigments were not clearly observed from the layer. The layers

appeared closely alike to the choroid around it. The RPE of group B, C and D all were not clearly visible. The absence of the RPE might be because RPE contains only one layer of cells which can easily be damaged by the high intensity light[2, 15].

A striking feature that was evident was the degeneration of the rod and cone layer outer segments in group B (exposed untreated), which is not seen in group C (prevention) and group D (therapy). What is seen is the very sharp distinction between the inner and outer segment of the rod and cone layer. As described by Xie[11] retinal light damage can be attributed to rhodopsin which is produced by the rod and cone outer segments. Hence the degeneration of the outer segment of the rod and cone layer of the animals in group B (exposed untreated). This also shows the ability of the fruit pulp of baobab to protect and treat the damage that was caused by the exposure to light.

CONCLUSION

1. LED Light exposure causes damaging effect on the retina of young Wistar rats.
2. The methanolic extract of the fruit pulp of *Adansonia digitata* has a protective effect on the histology of the retina of young Wistar rats.
3. The methanolic extract of the fruit pulp of *Adansonia digitata* has a therapeutic (healing) effect on the histology of the retina of young Wistar rats.

RECOMMEND

1. Larger animal model such as guinea pig and rabbit should be used for further research as they provide a larger eye size that allows for greater morphological studies.
2. Pigmented animal models should be used for further research to provide a more human like situation since albino rats are more susceptible to retinal damage by light.
3. Each animal should be exposed individually in a separate cage so as to ensure roughly equal amount of exposure to the light.

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