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Coconut oil protects against light-induced retina degeneration in male Wistar rats

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ABSTRACT

The retinoprotective effect of *Cocos nucifera* oil (CNO) was investigated. Twenty male Wistar rats weighing 140 g and 180 g were randomly divided into four groups comprising of five animals each. The control group received distilled water. Retinal degeneration was induced in the remaining three groups by exposing the animals to 5,000 lux of bright white light for two hours. Prior to the light exposure, the light model group (LMG) received distilled water for 14 days, low *Cocos nucifera* oil (LCNO) group received 5 ml/kg of CNO for 14 days, and the high *Cocos nucifera* oil (HCNO) group received 10 ml/kg of CNO for 14 days. The treatments continued for 7 days after exposure to light. On the eight day, the animals were euthanised and their retinas isolated. The right retinas and occipital cortices of the animals were prepared for histological evaluation while the homogenates of the left retinas were used for biochemical assay. The results show that CNO significantly ($p < 0.05$) reduced caspase-3 activity from 1.15 ± 0.054 ng/ml to 0.434 ± 0.095 ng/ml (LMG versus LCNO) and malondialdehyde concentration. There was no significant difference in the total antioxidant capacity in the retinas of the rats. However, LMG showed a significant increase in catalase activity. CNO was able to preserve the retinal morphology while LMG showed a distorted retinal layer and significant reduction ($p < 0.05$) in retina thickness. CNO was unable to prevent perineural vacuolations in the occipital cortices of the rats. In conclusion, *Cocos nucifera* oil produced retino-protective effect via anti-oxidative and anti-apoptotic mechanisms.

1. Introduction

Retinal degeneration is a multifactorial neurodegenerative disease characterized by progressive degeneration of photoreceptors/retinal pigment epithelium and results in irreversible central vision loss [1]. Examples of retinal degenerative diseases include retinal pigmentosa, Age-related Macular Degeneration (AMD), diabetic retinopathy.

In 1966, Noell et al [2] discovered that when the eyes of albino rats were continuously exposed to ambient light in the natural light spectrum, their retinas became irreversibly damaged. This discovery led many scientists to investigate the mechanisms involved in this light-induced retinal damage and how to protect the retina from such damage. Light-induced retina degeneration in laboratory animals is a well-established model used to understand the pathophysiology of retinal degeneration [3] and to identify measures that prevent retinal degeneration. Light induced retinal degeneration is an appropriate model for the study of retina degenerative diseases because light selectively affects the eyes without causing degeneration in other part of the brain.

Oxidative stress results from excessive production of reactive oxygen species that cause damage to cell components such as the mitochondria, lipids, proteins and DNA, eventually leading to cell death. Oxidative stress is known to be involved in vision-threatening diseases such as AMD [4], and light-induced retina degeneration pathophysiology has been attributed to oxidative stress [5]. Several studies have proven that administration of antioxidants can prevent light-induced retinal degeneration. However, most of these studies are *in vitro* [6,7]. Exposure of retinas to bright light can generate more free radicals than intrinsic protection mechanism can revert [8].

Apoptosis is the final manifestation of retinal degeneration. However, results are contradictory on the involvement of Caspase-3 in apoptosis of the photoreceptors. While some authors reported caspase-3 dependent apoptosis, others have reported a Caspase-3 independent mechanism [9,10].

Cocos nucifera oil (CNO) is derived from dried coconut fruit. Various beneficial effects of CNO have been investigated in experimental animals. Such beneficial effects include analgesic [11],

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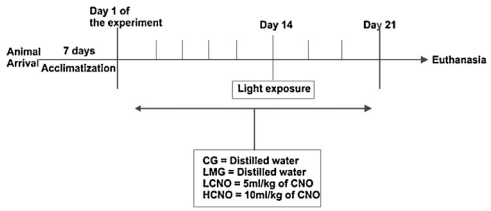

Fig. 1. Schematic representations of the experimental design.

anti-inflammatory [12], antimicrobial [13] and antioxidant [14] activities.

The antioxidant activity of CNO is attributed to biologically active components such as polyphenols, tocopherols, and medium-chained fatty acids notably lauric acid [15]. Also, CNO is one of the ketogenic diets [16]. Studies have confirmed that ketogenic diets offer neuroprotective effects against neurodegenerative disease through antioxidative and anti-apoptotic mechanisms [17].

CNO is made up of 50% lauric acid which represents its most effective active agent [18]. Other constituents include α-tocopherol [19] and fatty acids such as palmitic, oleic, linoleic, caproic, and caprylic acids [20]. Eight other phenolic compounds had been identified [21]. These are gallic acid, *p*-hydroxybenzoic, caffeic, ferulic, and syringic acids as well as catechins, epigallocatechins and epicatechins. The antioxidant activity of these phenolic substances has been proven [21]. The phenolic substances are able to scavenge free radicals.

Thus, CNO should be able to offer a protective effect on light-induced retinal degeneration, but no studies have yet investigated this possibility. Therefore, the aim of this study is to examine the possible protective effect of CNO on light-induced retinal degeneration in male Wistar rats. The study also aims to investigate the possible mechanism(s) of the protective effect of CNO.

2. Materials and methods

2.1. Chemicals and reagents

A Malondialdehyde assay pack, catalase assay kit and glutathione assay pack were obtained from Sigma Aldrich Ltd, Nigeria. The caspase-3 assay kit was obtained from Elab Science Biotechnology Co., Ltd, China. Other chemicals used were obtained from standard suppliers and they were of analytical grade.

2.2. Extraction of Cocos nucifera oil

The solid endosperm of mature coconuts was crushed and made into a viscous slurry. About 500 ml of water was added to the slurry and then squeezed through a fine sieve to obtain coconut milk. The resultant coconut milk was left for about twenty-four hours to facilitate the gravitational separation of the emulsion. Then the oil on top was scooped off and heated for about 5 min to remove moisture. The oil was finally filtered through a fine sieve and stored in a bottle at room temperature.

2.3. Experimental animals

Twenty adult Wistar rats weighing between 140 g and 180 g were used. They were housed in a standard cage in a 12-h light/12-h dark cycle. CNO was administered orally by the use of oral cannula.

2.4. Experimental design

The entire experimental design is shown in Fig. 1. The Wistar rats were randomly divided into four groups with each group containing five rats. The groups were:

Control group (CG), who only received distilled water

Light Model Group (LMG), who were exposed to 5,000 lux of white light for two hours and treated with distilled water.

The low *Cocos nucifera* oil (LCNO) group, who were exposed to 5,000 lux of white light for two hours and received 5 ml/kg of CNO.

The high *Cocos nucifera* oil (HCNO) group, who were exposed to 5,000 lux of white light for two hours and received 10 ml/kg of CNO.

The LCNO and HCNO groups were pretreated with CNO for 14 days before the light exposure and for another seven days after the light-induced retinal degeneration. The LM and control groups received distilled water throughout the experiment

Light exposure took place on the 14th day of the experiment. The rats were dark adapted for 18 h before exposure to constant light in a temperature-controlled reflective cage. Twenty minutes before exposure, their pupils were dilated with tropicamide eye drops. The rats were then exposed to two hours of cool white fluorescent light at a luminescence level of 5,000 lux as described by Wang et al. [22]. After exposure, they were returned to another 18 h of darkness as described by Wang et al. [22].

2.5. Biochemical analysis

At the end of the experiment, rats were euthanised by cervical dislocation and the eye balls enucleated. The retinas were isolated. The left retinas were homogenised and centrifuged, then the supernatants were assayed for malondialdehyde (MDA) concentration, total antioxidant capacity (TAC), catalase and gluthatione activities. Caspase-3 activity was used to determine photoreceptor apoptosis. The CNO used was also assayed for its phytochemical and lipid profiles.

2.5.1. Determination of caspase-3 concentration

The caspase-3 concentration was measured using Sandwich-ELISA method. The micro ELISA plate provided in the caspase-3 kit had been precoated with an antibody specific to CASP3. Standards or samples were added to the appropriate micro ELISA plate wells and combined with the specific antibody. Then a biotinylated-detection antibody specific for CASP3and an avidin-horseradish peroxidase conjugate were added to each microplate well successively and incubated. Free components were washed away. The substrate solution was added to each well. Only those wells that contain CASP3, biotinylated detection antibody and avidin-horseradish peroxidase conjugate appeared blue in colour. The enzyme-substrate reaction was terminated by the addition of a sulphuric acid solution causing the colour to turn yellow. The optical density was measured with a spectrophotometer at a wavelength of 450 nm ± 2 nm. This optical density is proportional to the concentration of CASP3. The concentration of caspase-3 in the samples was calculated by comparing the optical density of the samples to the standard curve.

2.5.2. Determination of lipid peroxidation in retinal tissue

The malondialdehyde concentration was assessed for lipid peroxidation following the assay method of Hunter et al. [23] and modified by Gutteridge and Wilkins [24].

2.5.3. Measurement of antioxidant markers

Gluthathione activity was assessed following the procedure of Beutler [25] and catalase activity was measured following the procedure of Aebi [26].

Table 1
Phytochemical constituent of CNO.

S/No.	Screened Phytochemicals	Constituents
1.	Terpenoids	++
2.	Steroids	+
3.	Anthraquinone	+++
4.	Tannins	–
5.	Saponins	–
6.	Flavonoids	–
7.	Cardiac	–
8.	glycoside Alkaloids	–

+++: Very strong;++: Strong;+: Weak; –: Absent.

2.6. Histological analysis

The right retinas and occipital cortices of the rats were separated and prepared for histological evaluation. The tissues were fixed in 10% formalin, dehydrated in graded alcohol, and later embedded in paraffin wax. The tissues were then cut into sections using a microtome. This was then stained with hematoxylin eosin. The slides were examined under a light microscope and photomicrographs taken.

2.7. Statistical analysis

Data were expressed as means ± standard errors of the mean. Statistical comparison was done using a one-way analysis of variance in SPSS (version 20). For intergroup comparison, *post hoc* testing was performed using the Bonferroni test with a $p < 0.05$.

3. Results

3.1. Phytochemical components of Cocos nucifera oil

The results from the analysis of CNO shows that the phytochemical components included terpenoids, steroids, and anthraquinone. However, the CNO did not have any tannins, saponins, flavonoids, cardiac glycoside or alkaloids (Table 1).

3.2. Lipid profile of Cocos nucifera oil

The lipid profile analysis shows that CNO has 60 mg/dl of triglycerides, 75 mg/dl of cholesterol and 41 mg/dl of high density lipoprotein (Table 2).

3.3. Effect of light-induced retinal degeneration and Cocos nucifera oil on caspase-3 concentration

Fig. 2 shows the mean concentration of caspase-3. There was a significant increase in caspase-3 activity in LMG compared to the other groups.

Table 2
Dose of lipids in every 1 dl of CNO.

S/No.	Lipid Component	Dose/Amount (mg/dl)
1.	Triglycerides	60
2.	Cholesterol	75
3.	HDL	41

HDL – High density lipoprotein.

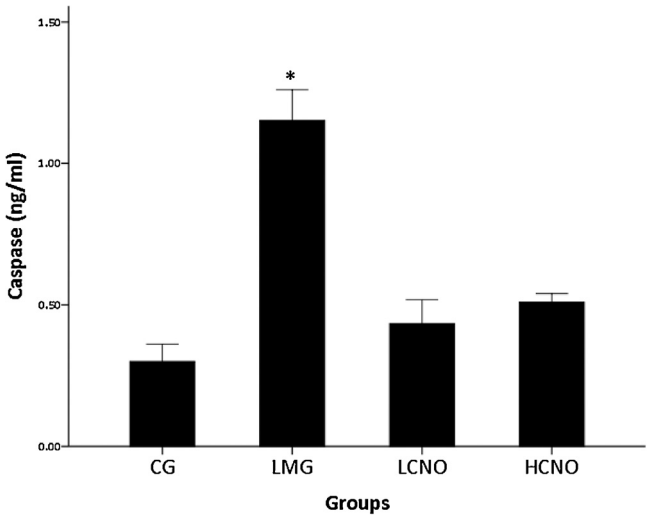

Fig. 2. Effects of CNO on Caspase-3 concentration in the retina of male Wistar rats. Each value is the mean ± S.E.M. of five Wistar rats; *significantly ($p < 0.05$) different compared with CG, HCNO and HCNO. CG = received distilled water throughout the experiment, the histoarchitecture and cellular integrity were normal (H&E, x100). LMG = received distilled water and exposed to light. LCNO group = received 5 ml/kg of CN oil for 14 days prior to light exposure and 7 days after light exposure. HCNO group = received 10 ml/kg of CN oil for 14 days prior to light exposure and 7 days after light exposure.

3.4. Effect of light-induced retinal degeneration and Cocos nucifera oil on malondialdehyde

Fig. 3 shows the mean concentrations of MDA. There was a significant increase in the MDA concentration of the LMG compared to the other groups. There was also a significant decrease in the MDA concentration in the HCNO group compared to the other three groups.

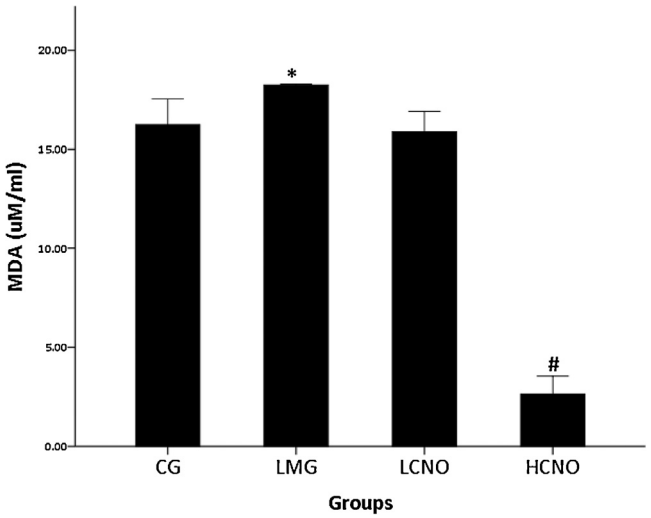

Fig. 3. Effects of CN oil on MDA concentration in the retina of male Wistar rats. Each value is the mean ± S.E.M. of five Wistar rats; *significantly ($p < 0.05$) different compared with CG, HCNO and HCNO. # Significantly ($p < 0.05$) different compared with CG, LMG, and LCNO. CG = received distilled water throughout the experiment, the histoarchitecture and cellular integrity were normal (H&E, x100). LMG = received distilled water and exposed to light. LCNO group = received 5 ml/kg of CN oil for 14 days prior to light exposure and 7 days after light exposure. HCNO group = received 10 ml/kg of CN oil for 14 days prior to light exposure and 7 days after light exposure.

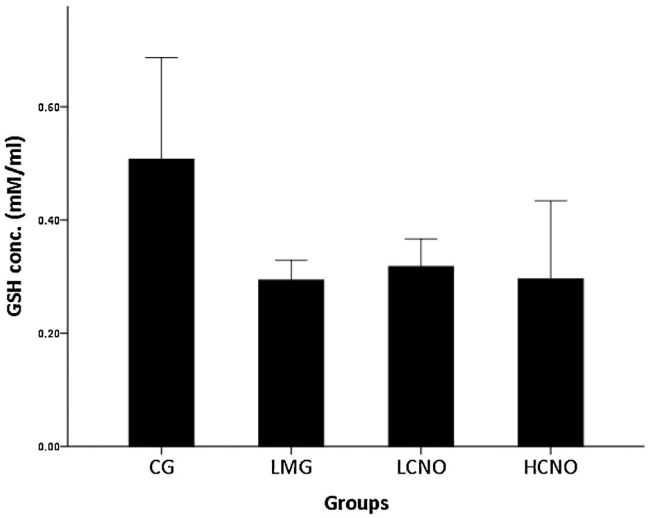

Fig. 4. Effects of CNO on GSH concentration in the retina of male Wistar rats. Each value is the mean ± S.E.M. of 5 Wistar rats; CG = received distilled water throughout the experiment. LMG = received distilled water and exposed to light. LCNO group = received 5 ml/kg of CN oil for 14 days prior to light exposure and 7 days after light exposure. HCNO group = received 10 ml/kg of CN oil for 14 days prior to light exposure and 7 days after light exposure.

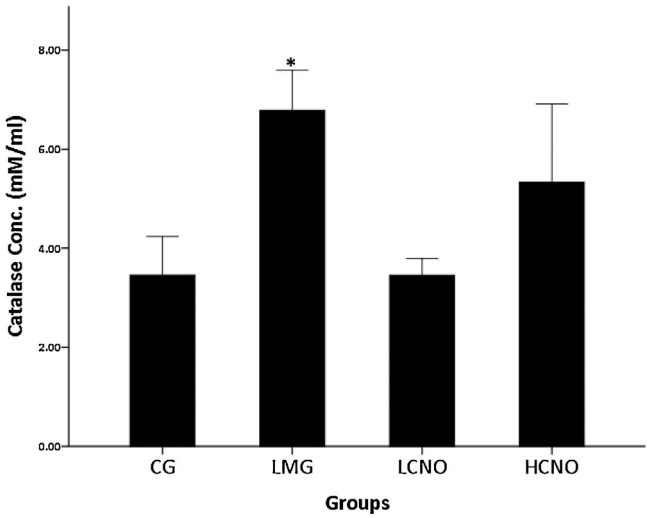

Fig. 5. Effects of CNO on Catalase concentration in the retina of male Wistar rats. Each value is the mean ± S.E.M. of five Wistar rats; *significantly ($p < 0.05$) different compared with CG, HCNO and HCNO. CG = received distilled water throughout the experiment, the histoarchitecture and cellular integrity were normal (H&E, x100). LMG = received distilled water and exposed to light. LCNO group = received 5 ml/kg of CN oil for 14 days prior to light exposure and 7 days after light exposure. HCNO group = received 10 ml/kg of CN oil for 14 days prior to light exposure and 7 days after light exposure.

3.5. *Effect of light-induced retinal degeneration and* Cocos nucifera *oil on glutathione peroxidase*

Fig. 4 shows the mean concentrations of glutathione. The mean concentrations of glutathione in the control, LMG, LCNO and HCNO groups were 0.427 ± 0.05 mM/ml, 0.294 ± 0.04 mM/ml, 0.318 ± 0.054 mM/ml and 0.381 ± 0.062 mM/ml, respectively. However, no significant difference was observed between the four groups.

3.6. *Effect of light-induced retinal degeneration and* Cocos nucifera *oil on catalase concentrations*

Fig. 5 shows the catalase activity of the groups. There was a significant increase ($p < 0.05$) in catalase activity in the LMG (6.79 ± 0.402 μM/ml) compared to the control (3.46 ± 0.384 μM/ml), LCNO (2.751 ± 0.45 μM/ml) and HCNO (4.477 ± 0.782 μM/ml) groups. These results show that the dosage of 5 ml/kg CNO, but not 10 ml/kg, reversed the increase in catalase concentration induced by light.

3.7. *Effect of light-induced retinal degeneration and* Cocos nucifera *oil on total antioxidant capacity*

Fig. 6 shows the TAC in the different groups. There was significant decrease in TAC in the LMG compared to the other groups.

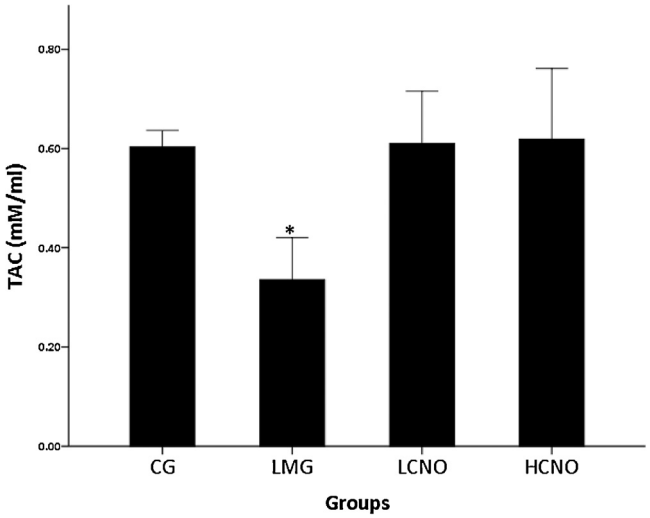

Fig. 6. Effects of CNO on TAC in the retina of male Wistar rats. Each value is the mean ± S.E.M. of five Wistar rats; *significantly ($p < 0.05$) different compared with CG, HCNO and HCNO. CG = received distilled water throughout the experiment., the histoarchitecture and cellular integrity were normal (H&E, x100). LMG = received distilled water and exposed to light. LCNO group = received 5 ml/kg of CN oil for 14 days prior to light exposure and 7 days after light exposure. HCNO group = received 10 ml/kg of CN oil for 14 days prior to light exposure and 7 days after light exposure.

3.8. *Effect of light-induced retinal degeneration and* Cocos nucifera *oil on retinal histology and thickness*

Fig. 7 shows the retinal histology with all the layers showing for all the groups. Fig. 8 shows the mean thickness of the retinas of each group. These means are 218.4 ± 5.58 μm, 113.42 ± 1.91 μm, 124.9 ± 2.22 μm, and 218.65 ± 4.61 μm for the control, LMG, LCNO and HCNO groups, respectively. There was no significant difference between the retinal thickness of the HCNO group and the control group. There was however a significant decrease in the retinal thickness of the LCNO group compared to the control and HCNO groups.

3.9. *Effect of light-induced retinal degeneration and* Cocos nucifera *oil on occipital cortices*

Fig. 9 shows the photomicrographs of the occipital cortices of the four groups. Perineural vacoulations were found in the LMG, LCNO, and HCNO groups.

4. Discussion

Studies on photochemical processes have suggested that the eye is most susceptible to visible light and UV radiation [27]. The photoreceptors and retinal pigmented epithelium are the parts of the

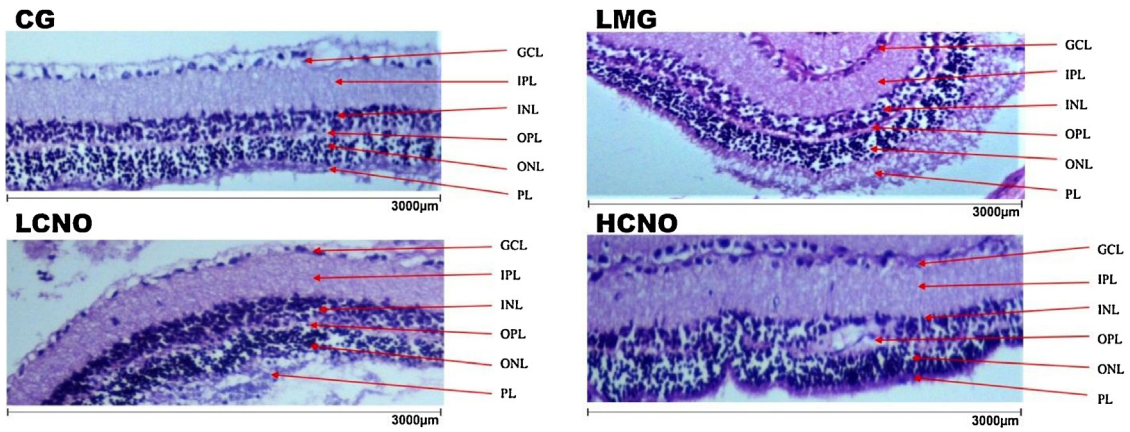

Fig. 7. Effects of CNO on retinas of male Wistar rats. CG = received distilled water throughout the experiment. There is intact retina layer. (H&E, x100). LMG = received distilled water and were exposed to light, the retina layer was intact but the thickness was reduced. (H&E, x100). LCNO = received 5 ml/kg of CN oil for 14 days prior to light exposure and 7 days after light exposure, the image shows distorted retinal layers with reduced retina thickness. (H&E, x100). HCNO = received 10 ml/kg of CN oil for 14 days prior to light exposure and 7 days after light exposure, the image shows intact retinal layers (H&E, x100). Number of animals per group were 5, but slides are representative samples. GCL: Ganglion Cell Layer, IPL: Inner Plexiform Layer, INL: Inner Nuclear Layer, OPL: Outer Plexiform Layer, ONL: Outer Nuclear Layer, PL: Photoreceptor Layer.

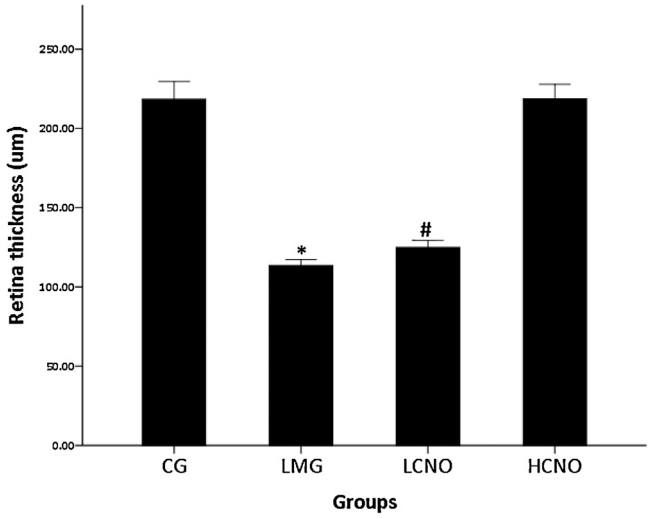

Fig. 8. Effects of CNO on retina thickness of male Wistar rats. Each value is the mean ± S.E.M. of five Wistar rats; *significantly ($p < 0.05$) different compared with CG, HCNO and HCNO. # Significantly ($p < 0.05$) different compared with CG, LMG, and HCNO. CG = received distilled water throughout the experiment. received distilled water throughout the experiment, the histoarchitecture and cellular integrity were normal (H&E, x100). LMG = received distilled water and exposed to light. LCNO group = received 5 ml/kg of CN oil for 14 days prior to light exposure and 7 days after light exposure. HCNO group = received 10 ml/kg of CN oil for 14 days prior to light exposure and 7 days after light exposure.

retina that are most susceptible to visible light [28]. Injury to these parts plays a crucial role in the pathogenesis of retinal degenerative diseases such as AMD [29,30].

Antioxidants have been shown to prevent retinal degeneration in light-induced models; however, most of these studies have been in vitro [31,32]. Numerous studies have shown that *Cocos nucifera* exhibits a wide range of effects including anti-inflammatory [12], hepatoprotective [33], antimalarial [34] and antioxidant [35] activities. Antioxidant activity is attributed to the presence of polyphenols. Considering their antioxidant property, polyphenols in CNO are expected to play a significant role in preventing retinal degeneration.

MDA is a biomarker for oxidative stress and has been implicated in the pathogenesis of light-induced retinal neurodegeneration [36]. In our study, light exposure significantly increased MDA concentrations in the LMG. This observation has also been noticed in previous research [37,38]. The high concentration of MDA noticed in the LMG is attributed to the high levels of polyunsaturated fatty acids present in photoreceptors. The LCNO and HCNO groups showed significant decreases in MDA concentration compared to the LMG. This shows that CNO exhibits a protective effect in retinal degeneration through antioxidative mechanisms at 5 ml/kg and 10 ml/kg by body weight.

Glutathione is a soluble antioxidant that detoxifies lipid peroxides and hydrogen peroxide by donating electrons to the peroxides thus reducing them to water and oxygen molecules [39]. Catalase is another endogenous antioxidant that can inactivate reactive oxygen species in cells [40]. During exposure to visible light, the endogenous antioxidant enzymes become activated so as to offer protection [41]. However, prolonged exposure to light can cause the production of free radicals in excess amounts that can then overwhelm intrinsic defence mechanisms and damage the retinas [42]. This overwhelming effect of reactive oxygen species is observed in the significant decrease in TAC in the LMG. There was also a significant increase in catalase activity in the LMG and shows that catalase is involved in combating oxidative activities in the retina.

There has been a long debate on the role of caspase-3 in the progression of light-induced retinal degeneration. Many researchers have reported that caspase-3 is involved in light-induced retinal degeneration [9,37] while others have argued that it is not involved [43,10]. In our study, caspase-3 was found to be involved in the pathogenesis of light-induced retinal degeneration given the observed significant increase in its concentration in the LMG. The caspase-3 activity observed in the control group is not considered abnormal because it is expressed in normal retinas; however, its role in maintaining the morphology of normal retinas is not exactly known [44]. The significant decrease in caspase-3 concentrations in the LCNO and HCNO groups, compared to the LMG, showed that CNO has anti-apoptotic activity. Thus, CNO may protect retinas through an anti-apoptotic mechanism.

This study showed that administration of CNO was able to preserve the structure of retinal morphology and its thickness. However, CNO was not able to prevent reduction in the thickness of the retinal layer at 5 ml/kg. In the LMG, there was disruption of the retinal layer and significant reduction in retinal thickness. Wang et al. [22] did not observe disruption in the retinal layer in their light model group, but they reported a significant reduction in retinal thickness. This same observation was also made by Wang et al. [37] in pigmented rabbits exposed to 18,000 lx of bright white light for two hours.

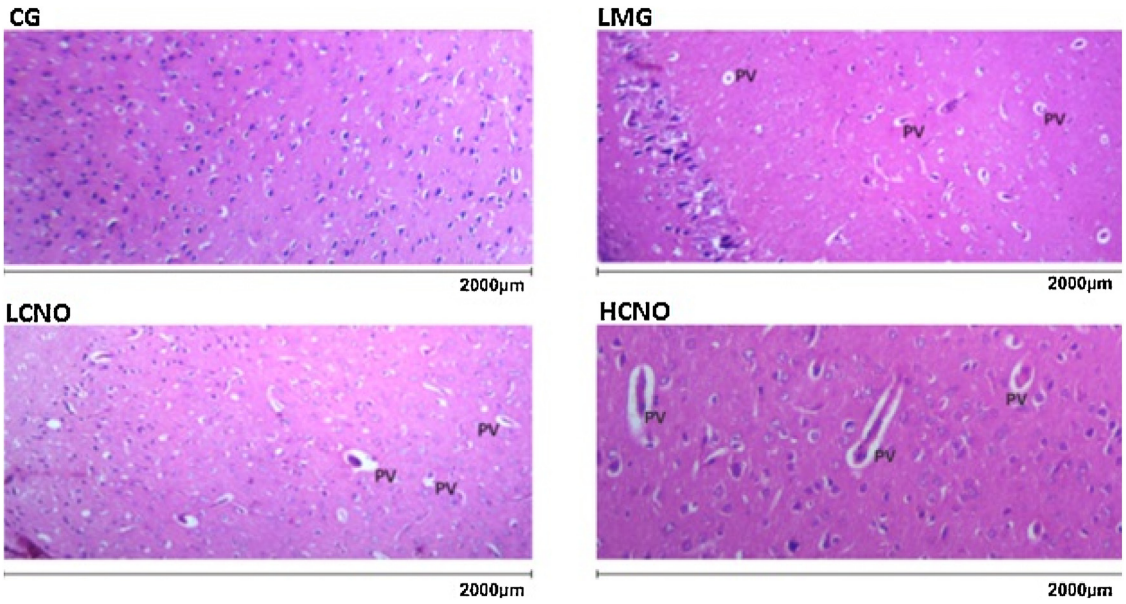

Fig. 9. Effects of CNO on occipital cortex of male Wistar rats. CG = received distilled water throughout the experiment, the histoarchitecture and cellular integrity were normal (H&E, x100). LMG = received distilled water and exposed to light, has occipital cortex with perineural vacoulation (PV). (H&E, x100). LCNO = received 5 ml/kg of CN oil for 14 days prior to light exposure and 7 days after light exposure, has occipital cortex with perineural vacoulation (PV). (H&E, x100). HCNO = received 10 ml/kg of CN oil for 14 days prior to light exposure and 7 days after light exposure, has occipital cortex with perineural vacoulation (PV). (H&E, x100). Number of animals per group were five, but slides are representative samples.

Light-induced retinal degeneration provides a retinal-specific injury unlike other chemical methods of inducing retinal degeneration [45]. This study also assessed whether the degeneration observed in the retina was reflected in the occipital (visual) cortices. No evidence of neurodegeneration was observed in the cortices of the control group which were not exposed to light. There were, however, perineural vacuolations in the occipital cortices of the LMG, LCNO HCNO groups. Therefore, it is reasonable to attribute the evidence of neurodegeneration observed in the occipital cortices of the LMG, LCNO and HCNO groups to the loss of photoreceptors in the retina. The caspace photoreceptor-dependent cortical degeneration theory has been promoted in various studies. Intense light exposure activates rhodopsin, which, in turn, triggers pathological changes [46] that include morphological and biochemical changes in the photoreceptors (the rod photoreceptor cell body, and especially in nocturnal rodents). These biochemical changes include an increase in cellular caspase activity that signifies (though not always) DNA degradation [47,48]. Also, within minutes of intense light onset, there is an increase in light-induced oxidative stress in the photoreceptors [49]. All of this can exacerbate visual cell death and lead to retinal degeneration [50] and consequential decrease in retinal thickness. A decrease in retinal thickness has been linked with cortical degeneration or atrophy [51,52]. Our present study shows that CNO treatments improved antioxidant defence, decreased caspase activity, improved retinal thickness, and protected against further cortical degeneration.

There are at least four possible mechanisms, apart from the antioxidant protective property, by which CNO may have exerted its protective effect on the retinas of the rats exposed to light. First, it may be as a result of the high concentration of beneficial lipids (fatty acids) in the CNO (Table 2). Photoreceptors, especially the rods' outer segment disc membranes, contain the highest levels of dietary docosahexaenoic acid compared to any other cellular organelle in the body [50]. These fatty acids are highly unsaturated and are vulnerable to oxidation by visible light [53] and molecular oxygen [54]. Hence, in light-induced retinal degeneration, CNO offers an optimal replacement for the dietary polyunsaturated fatty acids that were lost to light-induced lipid peroxidation. Apart from this, CNO contains saturated fats (Table 2) that are particularly resistant to lipid peroxidation [55]. Thus, these fatty acids can protect against the formation of free radicals. Second, the protective effect may result from increased blood ketone concentrations due to the consumption of coconut oil [56]. Increases in blood ketone levels are known to lead to increases in brain-derived neurotrophic factor (BDNF) [57,58]. BDNF is important for the growth, survival, and maintenance of neurons; research has shown that, by increasing BDNF concentrations, retinal cells can be protected from degeneration [45]. Third the anti-inflammatory properties of CNO may have an effect. Anti-inflammatory substances have been shown to protect against retinal degeneration [37]. Fourth, the terpenoids and anthraquinone that are present in CNO may provide a neuroprotective effect (Table 1). Studies have shown these neuroprotective effects of terpenoids and anthraquinone through antioxidative and anti-inflammatory mechanisms [59,60].

5. Conclusion

This research has shown that *Cocos nucifera* oil at a dose of 10 ml/kg of body weight can offer protection to retinal photoreceptor cells and histological integrity of the retina. This protective function is probably caused by the ability of *Cocos nucifera* oil to offer antioxidative protection and by inhibiting retinal cell apoptosis. Further investigations are required to detect the active compounds responsible for the probable anti-apoptotic action of CNO.

Conflict of interests

The authors have not declared any conflict ofinterest.

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