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Exploring the potential use of *Cannabis sativa* extract in ameliorating retinopathy

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Abstract
Cannabis preparations have been reported to have potential positive effects on retinal health and potential to ameliorate retinopathy or help in protecting the retina. This fact suggests that cannabis can improve vision health. A major component of cannabis that has been found to have such protective effects is cannabinoid. Cannabinoids have been demonstrated to have positive effects in lowering intraocular pressure, thus ameliorating or correcting glaucoma. It is thus very important to explore the potential use of cannabis preparation in correcting, ameliorating retinopathy. Research in this field are relatively sparse. To this end, the research involved testing the efficacy of various doses of cannabis ethanolic extract against sodium, iodate induced retinopathy in experimental animals. Thirty two Wistar rats were divided into four groups labelled: Group A, as the control group and the animals were fed ad libitum; Group B were administered a single dose of 50mg/kg body weight of sodium iodate to induce retinopathy; Group C were administrated lower dose of cannabis [200mg/kg body weight] while Group D were administered the higher dose of cannabis extract [500mg/kg body weight] after retinopathy was induced by a single dose of sodium iodate. Animals were treated as described for 12 days and thereafter sacrificed by cervical dislocation. The eyes were removed and processed for the H&E and the PAS histological and histochemical techniques respectively. Retinopathy, as induced resulted in retinal epithelial thinning; extensive loss of the ganglion layer of the retina; disruption of the pigment epithelium and relative distortion of the epithelial histoarchitecture. Cannabis could not effectively preserve the epithelium in the treated groups. Disruptions however were less extensive in the cannabis treated groups, to indicate that it had mild positive effects. Variation in dosage was not seen to produce significant difference in effects.

Keywords: Retina Retinopathy Cannabis Extract Wistar rat

1. Introduction
Retinopathy is an eye disease that occurs in the retina. It is a broad term used to describe damage to the retina of the eyes, which may further lead to vision impairment (Merriam-Webster). Retinopathy often refers to retinal vascular disease, macular degeneration, or damage to the retina caused by abnormal blood flow(Robbins, 2010). Frequently, retinopathy is an ocular manifestation of systemic disease as seen in diabetes or hypertension. Macular degeneration is a leading cause of irreversible blindness in the developed world. The retinal pigment epithelium (RPE) is a critical site of pathology (Zhou, *et al*., 2014) [21]. Retinotoxicity can be due to natural factors, induced, and due to complications such as trauma, hypertension and diabetes. Diabetic retinopathy accounts for about 5% of blindness worldwide and is designated a priority eye disease by the World Health Organization. Retinotoxicity can also be induced by endogenous and exogenous agents in laboratory animals. Sodium Iodate (NaIO3) has proven to be an effective way to induce retinopathy in animal models (Wang, *et al*., 2014) [18].
Cannabis also referred to as marijuana, is gotten from the preparation of the *Cannabis Sativa* plant. It is a popular recreational drug around the world, only behind alcohol, caffeine and tobacco. It is intended to be used as a psychoactive drug or medicine. Anecdotal reports suggest that one of the most psychoactive components of *Cannabis sativa* L. known as delta-9-tetrahydrocannabinol (THC) may have protective effects on the retina, at present, it remains unclear whether herbal cannabis, different natural or synthetic cannaboid CB1 receptor agonists or agents may have the best effect in retina preservation. At present, there

is no available effective treatment for retinopathy thus, more research is necessary to aid prevention through retinal protection.

The use of Cannabis as a possible treatment and prevention of retinopathy has not been extensively researched upon. This research focuses on explaining the underlying mechanism of this condition as well as finding out the role Δ9-THC has as a possible treatment to influence visual impairment.. While abundant experimental studies have illustrated the deleterious effects of cannabis use on cognitive functions (Kanayama, *et al*., 2004) [5] very few laboratory studies have examined its protective effects on the visual pathway.It is unclear whether herbal cannabis, different natural or synthetic cannaboid CB1 receptor agonists or agents may have the best effect in retinopathy, as positive and negative results have been recorded in the use of Cannabis in retina protection. The study primarily aimed at studying the effects the oral treatment of the ethanoic extract of *Cannabis sativa* will have on the visual pathway of the Sodium Iodate induced retinopathy Wistar rats.

Cannabinoids potential therapeutic uses have been reported (Landa L, Sulcova A., Gbelec P, 2006) [7]. Cannabis as a plant has been traditionally used for medicinal purposes over several centuries (Zuardi AW (2006) [22]: Kalant H (2001) [4]: It was reported to inhibit neurodegeneration in models of multiple sclerosis (Pryce *et al.,* 2003) [13]. The structure and function of cone and rod in the retina have been shown to benefit from cannabinoid agonist neuroprotective effects on retinal degeneration in autosomal dominant retinitis pigmentosa experimental rat models (Lax *et al*., 2014) [8]. More specifically, cannabis has been reported to have effects that are protective to the retina (Despina). In an attempt to ameliorate glaucoma in experimental conditions; cannabinoids effectively lowered the intraocular pressure (IOP); it also had neuroprotective effects (Nucci 2008; Tomida 2004; Marsicano, 2002) [12, 17, 10].

Materials and Methods

Animal Care and Housing

The animals were obtained from and were also kept and cared for in the Animal house in the Department of Agricultural Sciences, Babcock University, Ilishan-Remo, Ogun State according to the guide to the care and use of animals in research and teaching.

Twenty four male Wistar rats were used for the study. The animals were housed in plastic cages in a well aerated room with a 12-12 hr light/dark cycle, at normal room temperature. Rats were provided with rat chow and water *ad libitum*. Wood shavings were used as beddings for the animals. The beddings were changed every two days to avoid build-up of toxic ammonia levels. All rats were handled in accordance with the guidelines for animal research as detailed in the Guidelines for the Care and Use of Laboratory Animals.

Experimental Groupings

The 24 wistar rats were weighed at the end of the acclimatization period and divided into 4 groups; with each group having 6 rats each.

Group A: Control group- this group received only water and feed *ad libitum*.

Group B: Sodium iodate [NaIO3]- this group was administered sodium iodate only through the tail vein once.

Group C: NaIO3+low dose Cannabis- this group was administered sodium iodate only through the tail vein once after which a low dose of the ethanoic extract of cannabis was administered 24 hours later with an oral cannula.

Group D: NaIO3+high dose Cannabis- this group was administered sodium iodate only through the tail vein once after which a high dose of the ethanoic extract of cannabis was administered 24 hours later with an oral cannula.

The administration of drugs was done for 10 days using an oral cannula. The general view of the rats'eyes before, during, and after administration was monitored.

Experimental Etiquette

To improve animal welfare and scientific accuracy, The 3Rs of animal experimentation were considered. The experiments were refined to make sure animals suffered as little as possible and to make their living conditions in the lab comfortable.

NaIO3 Procurement, Preparation and Administration

Sodium Iodate was purchased from Sigma-Aldrich CO. LLC. Germany. The retinopathy rat models were established by tail vein injection of NaIO3 (50mg/kg) once in rats in the experimental groups. To calculate the dosage of Sodium Iodate to be administered to the rats, the average weight of all the rats was taken. 5mg of Sodium iodate was diluted in 0.1ml of distilled water which served as vehicle. 0.1 ml of sodium iodate solution contained 5 mg of the drug agent, and 1ml of the solution contained 50 mg of sodium iodate. The rats were placed on a sterile table. Their tails were cleaned with alcohol to enable easy viewing of the tail vein. The rat's tail was held between the thumb and the index finger of the left hand and the right hand containing the syringe was used to administer the Sodium iodate directly into the bloodstream through the tail vein.

Cannabis Procurement and Administration

After the study was approved by Babcock University Health Research Ethical Committee, and permission for the possession and purchase of Cannabis was granted by the National Drug Law Enforcement Agency, NDLEA. Cannabis leaves were obtained, blended to fine powder and soaked in absolute ethanol for 24 hours. The mixture was then filtered with a muslin cloth to obtain the filtrate- pure cannabis ethanoic extract. The filtrate was poured into a beaker, which was immersed in a water bath for 48 hours for dehydration, at a standard temperature of 40°C to avoid denaturation of the active ingredients. After 48 hours, the extract was scooped into glass petri dishes and further dried in an open air oven for two hours. The dry extract was then weighed and diluted in distilled water to obtain the ethanoic cannabis extract according to the standard research dosage, stored in plastic bottles and refrigerated.

Collection of Tissue Samples

At the end of the 10-day administration, the rats were sacrificed by cervical dislocation, just after weighing them. All protocols were observed and all precautions were taken before sacrificing the animals. The animals were then dissected on a dissecting board using a dissecting set to excise the eyes from their sockets. The excised organs were immediately fixed in 10% formal saline and sucrose solution in order to prepare for them tissue processing.

Results

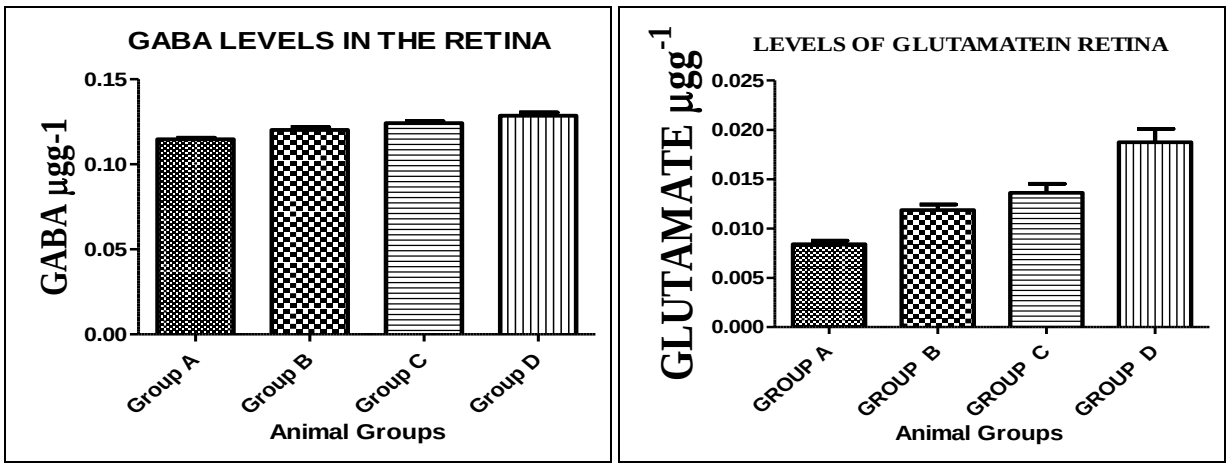

Fig 1: Charts shwoiing the leves of GABA and glutamate in the retina. The two vital retinal heirotratsmnitters were significantly increased in all treated groups relative to the control.

Histological Results

Photomicorgraphs preovide evidences of induced retinopathy in the trated groups and the nateure and extent of the effetcs of cannabis in an attempt to amiolarte the ncondition. Renipathy was sucssefully indcued in the Group B. This is evidient in the general disruption of the retinal histoacrhiteture. It is also obsverbable in the loss of the ganglion layer of the retina. The rertinal pigment epothelium was also distorted. Cannabis extract admistration did not effectively maeliorate the deelruoius effects of sodium iodate particlualry in terms of the loss of the ganglion layer, general dirtuption oif the retinal hsitoarchiteture and the diruotion of the pigment eputhelium.

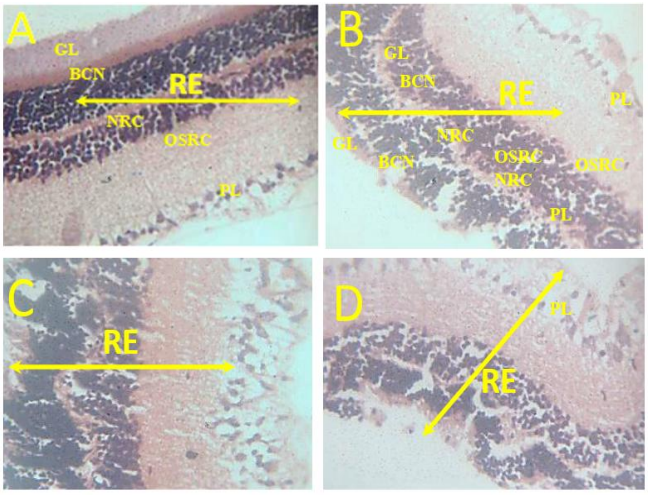

Fig 2: Photomicrographs of the retina of the Group A-D experimental animals [H&E A- X640]. Retinal most superficial layer is damaged and partly removed by sodium iodate. The structural integrity of the retinal layers is also disrupted. The administration of cannabis could not adequately ameliorate the structural damage of sodium iodate on the retina.

Histochemistry Results

Legend: [GL= Ganglion Cells; BCN= Bipolar Cell Nuclei; NRC= Nuclei of Rods and Cones; OSRC= Outer Segments of Rods and Cones; PL= Pigmented Layer; RE= Retinal Epithelium].

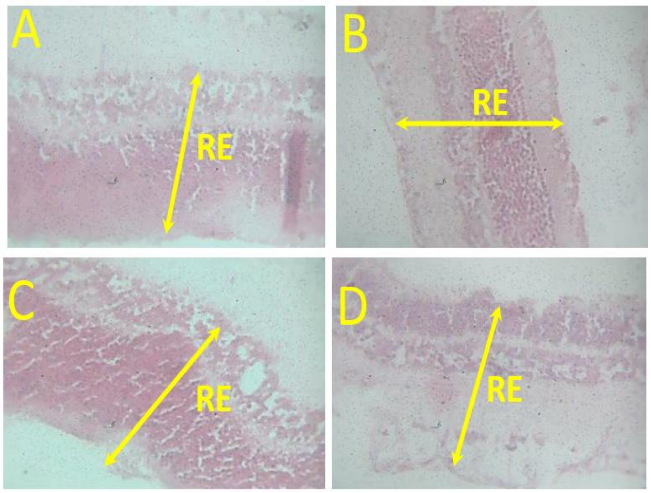

Fig 2: Photomicrographs of the retina of the Group A-D experimental animals showing the pattern of glycogen demonstration using the Periodic Schiff Acid method [PAS X640]. PAS demonstration of the retina shows mild aberrations that follow the pattern of structural disruptions.

Discussion

Retina Neurotransmitters Activities

The two neurotransmitters - GABA and glutamate were significantly raised in the treated groups relative to the control. Glutamate is arguably the most important neurotransmitter in the retina (Connaughton, 2007) [2] and it influences the synthesis and activities of GABA (Stryer, 1988) [16]. Therefore, in this case, glutamate expectedly influenced the activities of GABA. Glutamate administration on its own is deleterious to retinal integrity (1988). Glutamate levels and activities aberrations therefore are makers of retinal pathologies. Pharmacological blockage of glutamate transporter exposed inner retinal neurons to increased endogenous glutamate, and caused serious excitotoxic degeneration (Izumi, 2002) [19]. The aberrations in these neurotransmitters showed that sodium iodate caused retina toxicity and damage which could not be adequately ameliorated by cannabis.

General Structure of Tissues

Retinal epithelium when demonstrated using the haematoxylin and eosin staining technique presents a characteristic layered patterned with every layer having its characteristic feature that has a functional importance relative to retinal health and structural integrity. These with characteristic layers include: GL- Ganglion Cells; BCN- Bipolar Cell Nuclei; NRC- Nuclei of Rods and Cones; OSRC- Outer Segments of Rods and Cones; PL- Pigmented Layer. The control [Group A] is suitable to serve as a standard reference for the other experimental [treated groups] B-D. Retinal integrity in the context of the experiment is being interpreted in the context of the definition of each layer. Emphasis is being laid on the pigment epithelium or layer as it is often implicated in imminent or progressing retinal degeneration. PAS is being demonstrated as a marker of functional integrity relative to glycogen and mucosubstances material in the tissues.

Retinal epithelium is demonstrated in Group A with its characteristic layers suitable to serve as a standard reference for the other experimental [treated groups]. Retinal epithelium as demonstrated in Group B becomes relatively thin with its most superficial ganglion layer [s] removed. Pigment epithelium is also distorted. Retinal thickness/height is also relatively reduced. These signs are consistent with retinal degeneration that can lead to blindness based n compromised photoreception. he treatment administered to the Group C animals could only very mildly limit the extent of retinal degeneration. Retinal most superficial layer is damaged and partly removed. Retinal thickness is however relatively preserved compare to the previous groups; ganglion layer is also fairly preserved. The Group D photomicrographs show little persistent evidences of retinal disruption and a partial loss of the most superficial layer. Ganglion layer and retinal pigment layers are however fairly preserved. Retinal thickness is also relatively preserved. These observations show that the higher dose of the agent of intervention ameliorated more potently the sodium iodate induce retinal damage.

Special Features- PAS Expression

PAS demonstration of glycogen and mucosubstances material in the tissue helped to appreciate the functional integrity of the tissue relative to these substances and results are consistent with the structural observations as discussed above. PAS demonstration in the control [Group A] is normal and taken as the standard. It the group B, PAS demonstrates glycogen material in the tissue mildly poor and this is consistent with retinal degeneration. In Group C, PAS demonstration showed improvement over its demonstration in Group A. It is expressed in the retina in a way that is more similar to Group A, albeit, there is still persistent evidence of degeneration. In Group D, retinal thickness is however relatively preserved. PAS-glycogen expression is similar to its expression in Group A but less intense.

Generally, observations show that sodium iodate induced retinal degeneration. There are however not sufficient evidences to indicate that the administered cannabis extract at the various doses could effectively protect the retina as the effects were mild and might not halt degeneration of retina. They only could have mildly retarded the rate of degeneration or limit the extent.

While is has been argued that cannabinoids may help in ameliorating certain retinal disorders (Nucci *et al.,* 2007; Krishnan and Chatterjee, 2014) [11]; it should be noted that more studies are required about the specific modes of intervention if these reports are however practically applicable as suggested. Also, recommended use of synthetic cannabinoids as neuroprotective drugs for preventing and treating retinal diseases (Schwitzer, 2005) [15] might require the pharmacological modification of the isolated cannabinoids and not necessarily the ingestion or consumption of cannabis in its various preparations.

Conclusion

The use of cannabis ethnologic extracts was not adequately efficacious in ameliorating retinopathy induced by sodium iodate. Disruptions of the retinal pigment epithelium and overall retinal structural integrity and the loss of the innermost layers characterised sodium iodate induced retinopathy and remained largely persistent even in experimental animals that were subjected to interventions using the cannabis ethanolic extract.

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