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Short-Term Effect of Aqueous Mushroom (*Pleurotus tuber-regium)* Extract and Latanoprost on Intraocular Pressure Using Manometer

G.A. Akinlabi* and J.O. Ehidiamen

Department of Optometry, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria

*Corresponding author; Email: gaakinlabi@hotmail.com, Tel: +2348186146373

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ABSTRACT: *Pleurotus tuber-regium* also known as the King tuber mushroom is an edible gilled fungus native to the tropics, belonging to the *Pleurotaceae.* Recent studies have shown its ability to reduce intraocular pressure when compared with anti-glaucoma medications in animal models. Manometry has been the time proven and accurate method of determining intraocular pressure especially for research purposes. This study investigated the intraocular pressure reducing ability of *P. tuber-regium* when compared with Latanoprost (0.005%) in normotensive feline eyes. A total of 6 healthy medium-sized cats were used for this study. After an acclimatization period of 5 days, baseline intraocular pressure was taken. The animals were divided into two experimental groups (groups 1 & 2,) made up of three cats per group, with the right eye serving as the treatment eye and the left eye as the control for each group. For group 1, their right eyes were treated with aqueous mushroom extract (40 mg/ml, 3 drops t.i.d for 7 days) and group 2 with Latanoprost (0.005%, 1 drop o.d. for 7 days) topically. This was done after the intramuscular administration of general anaesthetic (Ketamine (500mg/10ml)) at 0.8 ml/kg. Intraocular pressure measurement carried out after the instillation of the drugs by cannulation of the anterior chamber using a mercury manometer. Mushroom extract decreased intraocular pressure by 12.8 % below baseline while Latanoprost decreased intraocular pressure by 26.3% on short term topical instillation with a p-value <0.05. There was no statistically significant difference for the control group at the pre and post treatment phase. Data obtained during this study showed that aqueous mushroom extract decreased intraocular pressure in the short term when compared with Latanoprost 0.005 %.

Keywords: *Pleurotus tuber-regium*, Latanoprost 0.005%, Manometry, Intra ocular pressure

Introduction

Mushroom extracts have gained popularity as remedies for a variety of ailments, including seasonal allergies, insomnia, cancer, the common cold and inflammation (Akpaja *et al*, 2003). *Pleurotus tuber-regium*, a popular edible mushroom, has been considered as a profound health promoting mushroom in traditional Chinese medicine (Huang *et al*, 2012). In addition to nutritive values, *P. tuber-regium* also exhibits some medicinal properties, including relief for stomach ailments, fever, asthma, smallpox, high blood pressure, and cancer (Oso 1975, Isikhuemhen *et al*, 2000). The fruit bodies of *P. tuber-regium* are rich in protein, while sclerotium is rich in fiber, especially non starch polysaccharides, mainly composed of bioactive *β*-glucans responsible for pharmacological actions. It possesses a lot of nutritive and medicinal value. Medicinal values include anti-tumor, immuno-modulatory, antioxidant, anti-inflammatory, hypocholesterolaemic, antihypertensive, antihyperglyceamic, antimicrobial, immuno-stimulatory and antiviral activities (Okolo 2017, Bonanno *et al* 2019, Gargano *et al* 2017, Maiti *et al* 2011). Administered topically,

the aqueous extract of *Pleurotus tuber-regium* dose-dependently reduces intraocular pressure (IOP) in dexamethasone-induced ocular hypertensive cats (Akinlabi *et al.*, 2008, 2009, Ebiigwai *et al*., 2012). For many years, IOP measurement has been the first line ocular parameter used in diagnosis and management of glaucoma. Instillation of an aqueous mushroom extract can cause a rapid or a slow exit of aqueous humor through the trabecular meshwork which is involved in most intraocular pressure readings (Akinlabi *et al.*, 2012).

Prostaglandins lower eye pressure by increasing the aqueous humor outflow through the uveoscleral pathway (Nordmann *et al.*, 2000). Normal cats exhibit a pronounced circadian rhythm in IOP with maximal values during the night and a gradual decline during the day (Del Sole *et al*., 2007). Diurnal fluctuation, elevations of IOP may occur only intermittently in some glaucomatous eyes, with as many as one-third of the measurements being normal (Ma *et al* 2016, Mottet *et al*., 2012). There exists a relationship between intraocular pressure and systemic blood pressure vis-à-vis systolic blood pressure (You *et al*., 2019).

Pooled data showed that latanoprost reduces mean diurnal IOP on average by approximately 30%, with a similar degree of intraocular lowering seen with bimatoprost and travoprost. Intraocular pressure may be affected in different species by several factors such as restraint, age, presence or absence of corneal disease and the time of the day in which tonometry was performed. Circadian rhythm has been described as a crucial factor when evaluating intraocular pressure in animal species. In most species investigated so far, intraocular pressure exhibits diurnal rhythms, but these variations differ both in amplitude, and whether there is an increase in intraocular pressure from morning to evening (Del Sole *et al.*, 2007). There exists some form of endogenous control of intraocular pressure in cats (Del Sole *et al.*, 2007, Liu *et al.*, 1991).

Manometry is an invasive technique that measures the real intraocular pressure as it is not affected by extraneous factors such as corneal thickness, scleral rigidity making it the most accurate method. It is used especially for research purposes under laboratory conditions to carry out continuous intraocular pressure measurement. It evaluates the effects of pharmacologic and physiologic manipulators and is useful in exploring the aqueous humor dynamics. It is also used for calibrating and validating the results of various types of tonometers on post-mortem human eyes and animal eyes (Danias *et al*., 2003). Due to its invasive nature, it is carried out under general anaesthesia to measure outflow facility using an intubated manometer with a cannulation needle.

Materials and methods

This was a prospective experimental study involving the use of six (6) healthy medium sized cats (1.3-2.0kg) of not more than 3 months in age. Six (6) healthy adult cats were purchased for this study and kept at the animal house of the department of Animal and Environmental Biology for five (5) days. Animals were properly fed with smoked fish during this period of acclimatization and their cages were cleaned regularly to prevent infection. Inclusion criteria was Six Healthy medium-sized cats (1.3-2.0kg) and the exclusion criteria was cats with any ocular pathology, cats with malaise and cats weighing outside the stipulated range**.**

Fresh *Pleurotus Tuber-regium* were harvested from the garden of the Department of Plant Biology & Biotechnology, Faculty of Life Sciences, University of Benin, Benin City. Extraction and concentration protocol described by Akinlabi *et al*. (2012) and Ebigwai (2012), was used.

The experiment was carried out in three (3) phases:

Phase 1: This involved acclimatization for five days in which the experimental animals were fed and observed for any signs of malaise, after which baseline intraocular measurement was carried out.

Phase 2: This involved division of the animal subjects into two experimental groups (3 cats each) with the right eyes as experimental eye and left eye as control.

- Group 1 – Aqueous mushroom extract (OD: 40 mg/1ml.3 gutt t.i.d x 1/52).
- Group 2 – Latanoprost (OD: 0.005%, 1 gutt dly x 1/52).
- Control - Untreated left eyes of Groups 1 and 2.

Fig. 1: Manometer glass tube mounted on a tripod stand.

Fig. 2. Anterior Chamber of the cat's eye being cannulated

Phase 3: Post treatment intraocular measurement was done for all the groups. Comparison was done between the intraocular pressure for the control and experimental group.
This was carried out using a mercury manometer. Glass tubes from University of Benin Chemistry laboratory was blown into a U tube shape using a glass blowing flame and then filled partially with mercury. After general anaesthesia with ketamine (1.0 ml/kg) a 25-gauge needle was inserted through the peripheral cornea into the anterior chamber of the cat's eye (the anterior chamber was cannulated in vivo with a 25-guage needle) (See Fig. 1 & 2).

Connected to the needle via polyethylene tubing is the mercury manometer. Manometers operate on the Hydrostatic Balance Principle: a liquid column of known height will exert a known pressure when the weight per unit volume of the liquid is known. Connecting one leg to an unknown pressure (IOP), the difference in column heights indicates the difference in pressures. The cannulating needle was removed ten minutes after insertion after intraocular pressure was determined followed by the instillation of antibiotic and non- steroidal inflammatory eyedrops to prevent any post cannulation infection or inflammation.
Results obtained from this study were analyzed using descriptive statistics and one-way ANOVA from the Statistical Package for Social Sciences Version 22.0. A p-value of 0.05 level of significance was used for this study.

Results

At baseline there was no significant difference in the two experimental groups in terms of intraocular pressure (p-value>0.05). This is shown in the graph below:

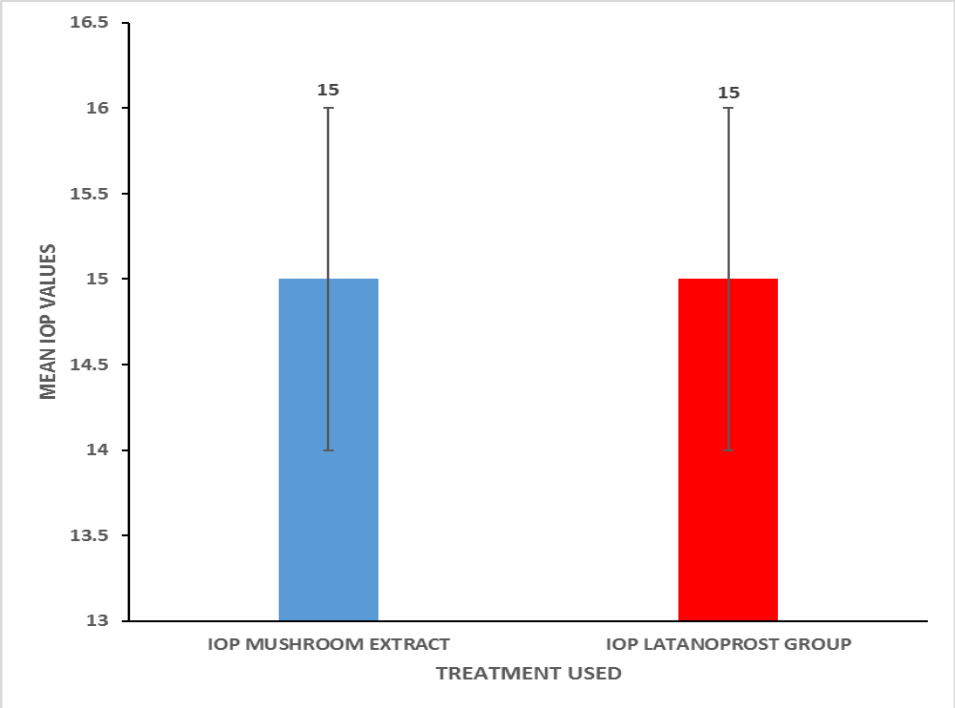

Fig. 3: Mean values of IOP at baseline. * Bars are not significantly different from each other ($P > 0.05$)
The mean value of three cats ± standard deviation of mean (S.D) were shown

At baseline, the mean values of the IOP following treatments from mushroom extract and latanoprost were both 15.0±1.0. From the ANOVA table, there was no significant difference in these two values at 0.05 significant level.
At the post treatment measurement phase, there was a significant difference in intraocular pressure in the two treatment groups (p-value 0.05) as shown in the graph below:

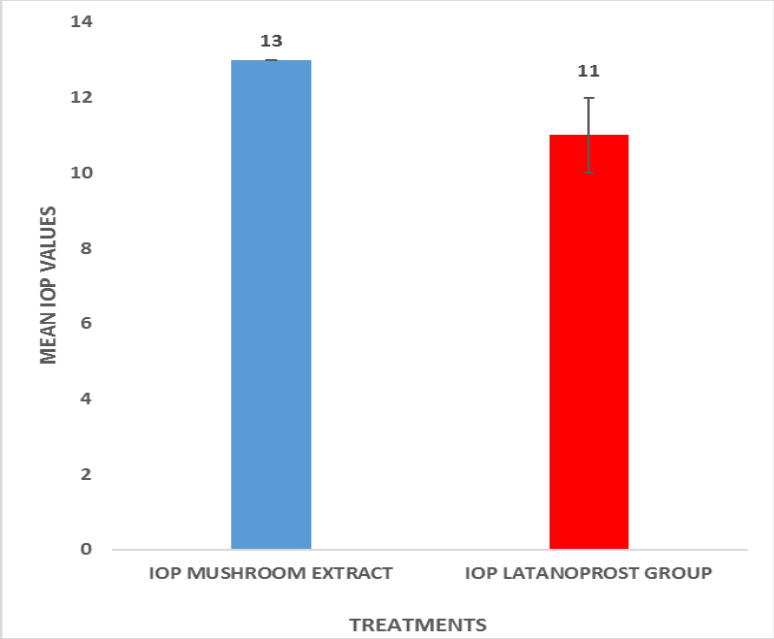

Fig. 4: Mean values of IOP following the treatments used in the experiment at post treatment stage. *Bars are significantly different from each other ($P < 0.05$). The mean value of three cats ± standard deviation of mean (S.D) were shown.

The mean values of the IOP following treatments from mushroom extract and latanoprost were 13.0 ± 0.0 and 11.0 ± 1.0 respectively. From the ANOVA table, there was significant difference in these two values ($P < 0.05$). There was a significant reduction seen more on the IOP mean values by the Latanoprost when compared to the mushroom extract. In other words, the Latanoprost treatment at this experimental stage possesses more significant potency in the reduction of IOP than the mushroom extract. At the pre and post treatment phases, intraocular pressure values for the control groups did not vary significantly from each other.

Discussion

This study investigated the short-term effect of aqueous mushroom (*P. tuber-regium)* and Latanoprost 0.005% on intraocular pressure of normotensive feline eyes using the manometric method. *Pleurotus tuber-regium* is known to have systemic effect among which is an anti-hypertensive effect. A functional relationship exists between blood pressure and intraocular pressure because low systemic blood pressure is known to affect ocular perfusion pressure. This implies low ocular perfusion pressure and choroidal blood flow would negatively affect blood flow to the ciliary body which contains the ciliary processes that secrete aqueous humor, thus causing a reduction in intraocular pressure. Latanoprost (0.005%) reduced intraocular pressure by 26.3% according to the data obtained during this study. This is in agreement with the work of other authors that says latanoprost reduces intra ocular pressure by 27-34% (Chew *et al.,* 2004, Mei-Ju *et al*., 2007, Sihota *et al*., 2004, McDonald *et al*., 2017). According to our study, aqueous mushroom extract decreased intraocular pressure by 12.8% at the post treatment phase in comparison with the baseline values implying that it reduced intraocular pressure on short term instillation in normotensive feline eyes. Thus, it has been demonstrated by this study that aqueous mushroom extract was half as effective as Latanoprost (0.005%) on short term topical administration readings from a mercury manometer. In an earlier study (Akinlabi *et al*., 2008), showed that when the mushroom extract was used for two weeks in steroid induced ocular hypertensive cats, it reduced their intraocular pressure by 45% when compared to steroid induced ocular hypertensive control, the concentration of the extract in this work was not stated. In another work, Akinlabi *et al*., 2009, administered 10 mg/ml of the mushroom extract, on the eyes of dexamethasone induced ocular hypertensive cats for four weeks, their intra ocular pressure was subsequently reduced by 30.4%. Our short-term result shows that

the effect of *P. tuber-regium* could be both dosage and duration dependent. Further research into a more effective dosage regimen and isolation of active constituent causing reduction of intraocular pressure is recommended. Clinical trials could also be carried as the mushroom extract has shown little or no ocular side effects on both long term and short-term topical administration.

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