STUDY ON EFFECT OF ETHANOLIC EXTRACTS OF *NEWBOULDIA LAEVIS*, LEAF, STEM AND ROOT ON CORNEO-CONJUCTIVAL INFLAMMATION AND INCREASED IOP IN RABBIT EYE

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DECEMBER, 2023

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A THESIS SUBMITTED TO THE DEPARTMENT OF OPTOMETRY, FACULTY LIFE SCIENCES, UNIVERSITY OF BENIN IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF DOCTOR OF OPTOMETRY (O.D) DEGREE

FACULTY OF LIFE SCIENCES

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BENIN CITY

EDO STATE

NIGERIA

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CERTIFICATION

This is to certify that this research project titled **(STUDY ON EFFECT OF ETHANOLIC EXTRACTS OF *NEWBOULDIA LAEVIS*, LEAF, STEM AND ROOT ON CORNEO-CONJUCTIVAL INFLAMMATION AND INCREASED IOP IN RABBIT EYE)** was carried out by (**ABULATAN OLUWASEUN)** in the Department of Optometry, Faculty of Life Sciences, University of Benin in partial fulfillment of the requirement for the Doctor of Optometry degree in the 2020/2021 academic session.

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DEDICATION

This work is dedicated to the Almighty God for his blessings in my life, his invaluable grace and provision throughout my stay in the university and for giving me the strength to complete this work. I also humbly dedicate this work to my parents Mr. and Mrs. ADENIYI ABULATAN, my siblings Dami, IB and Mayo, and my entire family for their unending love and support. More Love Less Ego.

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ABSTRACT

Extracts from different parts of the *Newbouldia laevis* plant (leaves, stem bark and roots bark), have been shown to possess antimicrobial, anti-malarial, antioxidant, nociceptive and anti-inflammatory properties. Previous experiments carried out have shown that the extracts from the leaf, stem bark and root bark have anti- inflammatory. But none of these studies have ascertained the IOP reducing effect of the extracts from the leaf, stem bark and root bark of the *Newbouldia laevis* plant in the eyes which was studied in this investigative work. This study aimed to investigate the effects of ethanolic extracts of *Newbouldia laevis* leaves, stems, and roots on physically induced corneo-conjunctival inflammation and increased intraocular pressure (IOP) in rabbits. Thirty (30) rabbits were used for this study and these rabbits were grouped into six ranging from group A through to group F. Each of the six experimental groups was treated with different extract (leaf, stem, or root) of *Newbouldia laevis*, while the control group was treated with sterile water. From the study, it was found that all three extracts (leaf, stem, and root) were effective in reducing corneal inflammation and increased IOP, and showed significant differences compared to the control group. ANOVA analysis used on the study showed the statical significant values ($p < 0.005$ was taken to be statistically significant) of the various treatments for corneal inflammation, ranging from group A- C having p values of: 0.002, 0.001, 0.265, respectively and induced IOP having p values of: 0.002, 0.002, and 0.001, respectively. During the course of this study, the leaf extract was found to be the most potent in the treatment of both the inflammation and induced IOP.

CHAPTER 1

1.0 INTRODUCTION

The term "traditional medicine" refers to the theories, beliefs, and practices that are unique to particular cultures and that are used to maintain health and to prevent, diagnose, treat, or improve physical and mental illness.In many nations, traditional medicine is frequently referred to as complementary or alternative medicine. 70% to 80% of people in the region have used herbal therapies as their primary method of healthcare, making them the most widely used type of traditional medicine.

In modern days, traditional medicine has been brought into focus for meeting the goals of a wider coverage of primary healthcare delivery not only in Africa, but also, to various extents, in all countries of the world and is the first-choice healthcare treatment for at least 80% of Africans who suffer from high fever and other common ailments (Treben, N.,1998). *Newbouldia laevis* is found in tropical Africa and it grows to a height of about 10 meters with a cauliferous habit.

Newbouldia laevis is popularly known as the tree of life or fertility tree in Nigeria (Ainooson, et al, 2009). *Newbouldia laevis* is commonly called African Border tree or boundary tree (Gbile and Adesina, 1987). It is called "Aduruku" in Hausa; "Ogirisi" in Igbo; *Kkhimi" in Edo and "Akoko" in Yoruba languages (Ogunlana and Ogunlana, 2008).

Newbouldia laevis bark is rinsed cleaned then masticated and swallowed to aid in healing stomach pain, and toothache. (Lewis and manony, 1977, Amechina. 2003). The extracts of *Newbouldia laevis* plant are used for the treatment of elephantiasis, rheumatic swellings, syphilis, constipation and pile. It is also useful for the treatment of ear ache, sore foot, chest pain, epilepsy and convulsion (Akunvili. 2000). The leaf, stem and fruits have been used for stomach ache and

for wound dressing (lwu, 2000). *Newbouldia laevis* plant is a multipurpose one and its leaves, fruits and flowers, stem bark and roots have various medicinal uses. Studies by (Oliver-Bever, 1986) showed that the leaf and root bark contain flavonoids, saponins, quinines, terpenes and steroids. The aqueous and ethanolic leaf extracts of *Newbouldia laevis* showed contraction on uterine effects (Bafor and Sanni, 2009). More so, like a lot of medicinal plants, many of the uses

and possible adverse effects of the plant by traditional medicine practitioners have not been investigated. Hence, this research investigated possible effect of the leaf, stem and root bark extract on ocular inflammation and intra-ocular pressure.

Figure 1.1: *Newbouldia laevis* tree plant

1.1 Background Information

1.1.1 Cornea And Vision

The cornea is a clear, avascular structure located at the front of the eye that helps to focus light on the retina. It measures approximately 11-12mm horizontally and 9-11mm vertically, and contributes about two-thirds of the eye's total refractive power. The cornea is sensitive to touch, temperature, and chemical stimuli and is vital for vision because of its transparency. It is responsible for two-third of the eye's total refractive power of 43D with a refractive index of 1.376. It does not contain blood vessels and receives nutrients through diffusion from the tear film on the outside and the aqueous humor inside the eye.

1.1.2 **Cornea Histology**

Histologically, the cornea has five distinct layers (Khurana 2003). From antero- posterior these are;

- Epithelium
- Bowman's membrane
- Substantia propria (Stroma)
- Descemet's membrane
- Endothelium

- Corneal Epithelium: The cornea is composed of a thin, multicellular layer of stratified squamous cells that connect with the epithelial cells of the bulbar conjunctiva at the limbus. It has about 5-6 layers of cells that are continually shed and regenerated through the multiplication of cells in the basal layer. The cornea is a fast-growing, easily

regenerated tissue that is kept moist by tears. It serves as a barrier to chemicals, water, and microorganisms, and has some immune functions due to the presence of Langerhans's cells.

- Bowman's Layer: it is a tough layer composed of collagen, laminin, pelican that

protects the stroma. It is not a true elastic membrane but simply a condensed

superficial part of the stroma. It is considerably resistant to infection but once destroyed, does not regenerate. The Bowman's layer helps in maintaining corneal shape.

- Corneal Stroma (Substantia Propria): The stroma is the middle layer of the cornea, about 0.5mm thick, and makes up about 90% of the total thickness of the cornea. It consists of collagen fibrils (lamellae) in a hydrated matrix of proteoglycans. The cornea stroma provides mechanical strength, helps maintain corneal transparency, and serves as the main refracting lens of the cornea.
- Descemet's membrane: The Descemet's membrane is a thin layer located at the back of the cornea that serves as the support structure for the endothelial cells. It is composed of collagen and glycoproteins and is bound to the stroma posteriorly. The Descemet's membrane is resistant to chemical agents, trauma, and pathological processes and is able to maintain the structural integrity of the eye for an extended period of time. Unlike the Bowman's layer, it has the ability to regenerate.
- Endothelium: The endothelium is a single layer of flat, polygonal cells located at the back of the cornea. It helps maintain corneal clarity by removing excess water from the cornea stroma. The endothelium is a simple squamous or low cuboidal monolayer of cells that is approximately one micrometer thick and rich in mitochondria.

1.1.3 Corneal Inflammation

Corneal inflammation (keratitis) is a condition where the corneal is inflamed and shows the following characteristics of corneal oedema, cellular infiltration and ciliary congestion. It may be superficial or deep and can lead to corneal ulcer when not properly treated. The five cardinal signs of inflammation are;

- Pain
- Redness
- Immobility
- Swelling and
- Heat

Keratitis has many potential causes which includes; infections (such as bacteria, virus, fungi, contact lens induced acanthamoeba keratitis), physical or chemical trauma, dry eyes, etc. The symptoms include; eye pain, blurred vision, photophobia, tearing and red eye.

Once the corneal epithelium has been invaded by the inflammatory agents, the sequences of pathological changes that occur include the following;

- Infiltration
- Active ulceration
- Regression
- Cicatrization

Figure1.2 : 3mm slit knife

1.1.4 Intra Ocular Pressure

Intra ocular pressure is the measurement of the amount of fluid pressure (aqueous humor) inside the eye. It is determined by the production and drainage of the aqueous humor by the ciliary body and drainage via the trabecular meshwork and uveoscleral outflow. The normal IOP of humans is between 11-21mmHg. There is diurnal variation of IOP of about 3-6mmHg for normal eyes and even more for the eyes of those with glaucoma.

1.1.5 Composition Of Aqueous Humor

The aqueous humor is a transparent, watery fluid similar to the plasma but containing a lower protein concentration. it has a pH of 7.4 and osmolarity of 304. It is made up of;

- 98% water
- Amino acids
- Electrolytes (sodium, potassium, calcium, magnesium, chloride, phosphate)
- Ascorbic acid

- Glutathione
- Immunoglobulin

1.1.6 Mechanism of Production of Aqueous Humor

The production of aqueous humor follows a daily cycle and is produced by the ciliary processes, which contain a double layer of epithelial cells and a supply of capillaries. Aqueous humor is formed through active secretion in the double-layered ciliary epithelium, ultrafiltration, and simple diffusion.

- Ultrafiltration: The substances in the plasma pass out from the capillary wall and

accumulate behind the non-pigmented epithelium of the ciliary process. It is a pressure

dependent movement along a pressure gradient.

- Simple diffusion: Diffusion is the passive movement of ions across a membrane related to charge and concentration. Sodium helps with the movement of water into the posterior chamber.

Drainage of aqueous humor

The flow is from the posterior to the anterior chamber through the pupil. From the anterior

chamber, it is drained by two major routes;

- Trabecular (conventional) outflow
- Uveoscleral (unconventional) outflow

1.1.7 Significance Of The Intraocular Pressure

Elevated intraocular pressure (IOP) can lead to a range of ocular complications, including glaucoma. Glaucoma is a group of disorders characterized by progressive optic neuropathy, which can cause specific changes in the optic disc and irreversible visual field defects, and is often associated with high IOP. High IOP puts mechanical stress on the lamina cribosa, disrupting blood flow and causing a shortage of growth factors (neurotrophins) that support the survival of retinal ganglion cells. While high IOP is the most common risk factor for glaucoma, it is not the only one. Ocular hypertension refers to consistently elevated IOP without any associated glaucoma damage, while normal or low-tension glaucoma (NTG/LTG) refers to typical changes in the optic disc and/or visual field defects that occur with normal or low IOP.

1.1.8 Variation of IOP

A healthy eye should have roughly consistent intraocular pressure at all times. This demonstrates how distinctive and efficient the pressure controlling system is. This is due to the fact that the rate of aqueous generation and drainage is roughly similar. Therefore, a change in the equilibrium between aqueous production and outflow is typically to blame for an unexpected increase in intraocular pressure. The intraocular pressure changes throughout the day (diurnal fluctuation), and is often highest just after awakening and lowest in the late afternoon. In glaucomatous eyes, where it can reach up to 40mmHg in fluctuation, this diurnal variation can range from 3-4mmHg and considerably higher. Other differences include changes in blood pressure, pulse, and breathing that have an impact on the arterial pulse might vary by up to 2-3 mmHg due to the intraocular pressure expanding the ocular coatings and vessel walls. The systolic phase of the pulse causes the arteries in the eyes to dilate, which raises intraocular pressure.

1.1.9 Factors that Influence Intraocular Pressure

- Trauma
- Heredity
- Sex
- Diurnal variation
- Postural variation
- Blood pressure:
- Osmotic pressure of blood
- Age
- Myopia

1.1.10 Measurement of Intraocular Pressure

Intraocular pressure is determined by the production and drainage of aqueous humor by the ciliary body and its drainage via the trabecular meshwork and uveoscleral outflow. The reason for this is because the vitreous humor in the posterior segment has a relatively fixed volume and thus, does not affect intraocular pressure regulation.

An important quantitative relationship is provided by Aptel, et al. (2016) below:

$Po=F-UV/C+Pv$

Where:

Po is the IOP in millimeters of mercury (mmHg)

F the rate of aqueous humor formation in microliters per minute (uL/min)

U the resorption of aqueous humor through the uveoscleral route (in uL/min)

C is the facility of outflow in microliters per minute per millimeter of mercury

(uL/min/mmHg)

P the episcleral venous pressure in millimeters of mercury (mmHg).

The above factors are those that drive IOP.

1.1.11 Instrument for measurement of Intraocular Pressure

Intraocular pressure of the eye is measured with an instrument; Tonometer. The process of measuring intraocular pressure with a tonometer in clinically known as tonometry. Tonometry is basically an objective measurement of the intraocular pressure of the eye, based mostly on the force required to flatten the cornea or cornea indentation produced by a fixed force. There are two main methods of tonometry, which

- Contact tonometry and
- Non-contact tonometry

Contact tonometry can be sub divided into

- Indentation tonometry: This method of measuring intraocular pressure is based on the

principle of indentation. An example of a tonometer that operates on this principle is the Schiotz tonometer. It has a plunger with a preset weight which indents the anaesthetized cornea as the patient looks upward lying in a supine position. The intraocular pressure is determined by the area indented by the fixed weight. The amount of indentation is read

off on a scale and the reading is converted into mmHg with the use of a special table. Most times this method is not reliable because of the cornea and scleral rigidity and to a lesser extent, curvature of the cornea.

- Applanation tonometry: This method of tonometry operates on Imbert-fick principle. The intraocular pressure is proportional to the pressure applied to the globe (cornea) and the thickness of the walls of the globe (cornea thickness). This method of measuring intraocular pressure is more accurate compared to the indentation tonometry.

The principle of Applanation also applies to the non-contact tonometry; however, instead of using prisms, the center of the cornea is flattened by a jet of air. A section of the cornea acts optically as a mirror. This makes sure that the reading is taken at a specific distance from the cornea. The time required to sufficiently flatten the cornea relates directly to the level of intraocular pressure. This method is easy to carry out and does not require topical anesthetics.

Types of Tonometry

- Perkins (Applanation tonometry): this uses the Goldman prism adapted to a small source of light. It is handheld and does not require a slit lamp.
- Goldmann (Applanation tonometry): this has double prism with a diameter of 3.06mm. It is known to be very accurate, although there may also be some errors due to

inappropriate fluorescein staining pattern.

- Schiotz (Indentation tonometry): this has a plunger that is used to indent the cornea. Intraocular pressure is determined by the amount of weight required to flatten the cornea. This is converted into millimeter of mercury using a conversion table.

- Pulsar air puff tonometer: this tonometer does not make contact with the cornea when being used for measurement. It uses a puff of air to flatten the cornea. It is considered as the best method of measuring intraocular pressure in children and patients who had Lasik surgery.

- Tono-pen handheld electronic contact tonometer: This is gently placed against the tear film and the pressure reading appears on the digital read out simultaneously to a faintly audible beep.

- Pneumo-tonometer contact device: this is operated similarly to the handheld tono-pen; however, due to its large size, it is not portable. It also requires a constant supply of gas. This technology is considered old and has largely been replaced by the tono-pen.

Pascal Dynamic Contour tonometer (DCT): this type of tonometer uses a built-in sensor tip with a solid-state pressure sensor that matches the corneal curvature.

1.1.12 GLAUCOMA

Glaucoma is a set of eye conditions that cause damage to the optic nerve, a vital structure that carries visual information from the eye to the brain. This damage is often associated with high

pressure within the eye, but it can also occur at normal eye pressure levels. Glaucoma can affect people of any age, but it is more common in older adults and is one of the leading causes of blindness in those over the age of 60. One of the most concerning aspects of glaucoma is that many forms of the condition have no noticeable symptoms, so the gradual loss of vision may not be noticed until the condition has progressed to advanced stages.

Risk factors

- Prolonged increased intraocular pressure
- Black race, Asian heritage
- History of glaucoma in the family
- medical conditions, such as diabetes, elevated blood pressure
- Corneas with Thin center
- High myopia or hyperopia
- Use of corticosteroid eyedrops for a prolong period of time

Types of Glaucoma

- Open-angle glaucoma: This form of glaucoma is called primary open-angle glaucoma (POAG). It is the most common type of glaucoma and occurs when the drainage angle between the iris and cornea remains open, but other parts of the drainage system are not functioning properly. As a result, eye pressure may gradually increase over time. If left untreated, POAG can lead to progressive damage to the optic nerve and vision loss. It is

important to have regular eye exams to detect glaucoma early, as it is typically asymptomatic in the early stages and treatment is most effective when started early.

- Angle-closure glaucoma: Angle-closure glaucoma (also known as acute angle-closure glaucoma or narrow-angle glaucoma) is a form of glaucoma that occurs when the iris bulges forward and partially or completely block the drainage angle between the iris and cornea. This blockage prevents fluid from circulating through the eye, leading to an increase in eye pressure. Angle-closure glaucoma can develop suddenly or gradually.
- Normal-tension glaucoma: It is not fully understood why the optic nerve becomes damaged in some cases of glaucoma even when eye pressure is normal. There are several theories about why this may occur, including the idea that the optic nerve may be more sensitive or have reduced blood flow. Reduced blood flow to the optic nerve could be caused by the accumulation of fatty deposits in the arteries (atherosclerosis) or other conditions that affect circulation. Some research suggests that these factors may contribute to the development of normal-tension glaucoma, a form of glaucoma in which eye pressure is within the normal range but the optic nerve is still damaged.
- Congenita glaucoma: Pediatric glaucoma is a form of glaucoma that affects children. It can be present at birth (congenital glaucoma) or develop in the first few years of life. Pediatric glaucoma may be caused by a variety of factors, including blockage of the drainage channels in the eye, injury, or an underlying medical condition. It is important to diagnose and treat pediatric glaucoma as early as possible, as the condition can cause vision loss if left untreated.

Treatment of Glaucoma

- Trabeculectomy
- Laser therapy
- use of Oral medication usually a carbonic anhydrase inhibitor.
- Prescription eye drop such as
    1. Prostaglandins: they increase ocular fluid outflow e.g., latanoprost
    2. Beta blockers: they reduce IOP by reducing the fluid production of the eye e.g., betaxolol
    3. Alpha-adrenergic agonists: they reduce IOP by reducing the fluid production of the eye e.g. brimonidine.
    4. Carbonic anhydrase inhibitors: they lower IOP by reducing fluid production in the eye e.g., dorzolamide
    5. cholinergic agents: e.g., pilocarpine.

1.2 STATEMENT OF PROBLEM

Newbouldia laevis plant have its anti-inflammatory effect on various systemic pathology using animal models in various studies carried out. However, its anti-inflammatory effect and intra ocular pressure reducing properties on ocular tissues has not yet been described in the right measured dosages, and there seems to be a few documented researches to describe its effect on intraocular pressure. The cornea's shape and clarity have a significant impact on how clear your vision can be (Nishida et al., 2021). Increased intraocular pressure, and anterior uveitis are a few of common conditions that have debilitating effects on vision if poorly managed or left untreated. Corneal opacity which is an expected result of corneal lacerations, is the 5th most common cause

of blindness in the world with a 3.46% prevalence (Saka, 2017), (Hashemi et al., 2022). This study aims to investigate the right dosage to give the best anti-inflammatory and ocular hypotensive effect of *Newbouldia laevis* plant leaf, stem bark and root bark extract using animal models.

1.3 AIMS AND OBJECTIVES

1.3.1 AIMS OF STUDY

The study aims to determine the effect of *Newbouldia laevis* plant leaf, stem bark and root bark ethanoic extract on intraocular pressure and ocular inflammation.

1.3.2 OBJECTIVES OF STUDY

1. To determine the effect of *Newbouldia laevis* plant leaf, stem bark and root bark on intraocular pressure in the New Zealand rabbit's eye.
2. To determine the effect of *Newbouldia laevis* plant leaf, stem bark and root bark on inflammation in the New Zealand rabbit's eye.

1.4 HYPOTHESES

Null Hypotheses (Ho1): The *Newbouldia laevis* plant leaf, stem bark and root bark has no effect when used separately on intraocular pressure.

Null Hypotheses (Ho2): The *Newbouldia laevis* plant leaf, stem bark and root bark has no effect when used separately on inflammation.

1.5 SIGNIFICANCE OF STUDY

Increased intraocular pressure (IOP) is a leading risk factor for the development and progression of glaucoma (Goldberg, 2003). Iyamu and Ahmed (2004) had stated in their study that blindness is a significant burden to society in that the cost of loss of productivity and of rehabilitation and education of the blind is very high and increasing. The soft and effective use of resources for the prevention of blindness will provide enormous savings in both money and human suffering. Data obtained from population-based surveys (PBS) indicate that glaucoma is the second leading cause of blindness, accounting for 8% of blindness among the 39 million people who are blind world- wide (Pascolini and Mariotti, 2010). In Africa, glaucoma accounts for 15% of blindness and it is the region with the highest prevalence of blindness relative to other regions world-wide (Resnikoff et al. 2004).

Most ocular inflammation always and usually take the pattern of uveitis. Uveitis could be defined as inflammation of the uveal tract with or without the involvement of adjacent ocular structures (Abdulaal et al., 2015). It is a major cause of visual loss both in developed and developing nations of the world (Gameiro, et al., 2017).

Glaucoma is a leading cause of irreversible worldwide. This study would aid to increase the available options for the management of increased IOP with a cheaper option in the best dosages. Ocular inflammation is a condition that can lead to visual impairment if not managed immediately, Ocular inflammation when left to fester usually result in a sequela of conditions that may result in vision loss. This study would aid to increase the available options for the management of inflammation and IOP reduction with a cheaper option in the best dosages.

This study will therefore create awareness about the efficacy of *Newbouldia laevis* in the

remediation of ocular inflammation and high intra ocular pressure. Thus, enabling an affordable and safe alternative drug regime.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Anti-Inflammatory Effect Of *Newbouldia Laevis*

Various study done with *Newbouldia laevis* suggest a fair amount of potency in its pharmacological properties. A study was carried out by Udeozo et al., (2014) to determine determine the phytochemical, anti-inflammatory and acute toxicity properties of *Newbouldia laevis* flower. Acetic acid-induced writhing in mice and formalin test in rats were used to carry out this study. The ethanolic extract of the *Newbouldia laevis* flower caused a significant decrease ($p<0.05$) in the induced conditions which was not a dose dependent inhibition on acetic acid induced writhing and the neurogenic pains which was induced by formalin. The *Newbouldia laevis* flower extract at the doses of 25, 50 and 100 mg/kg showed 59. 71 and 47% inhibitions of the abdominal construct in mice respectively.

Chukwuma and Nwachukwu (2016) conducted a study to investigate the antimicrobial properties of *Newbouldia laevis* leaves and the potential of these leaves to treat wound infections when purified to an appropriate pharmacological level. The researchers used a modified agar well diffusion method to test the antimicrobial activity of the plant extract against a range of microorganisms, including Escherichia coli, Klebsiella pneumonia, Proteus sp., Staphylococcus aureus, Pseudomonas aeruginosa, Staphylococcus epidermidis, and Candida albicans. Phytochemical analysis of the ethanolic extract revealed the presence of flavonoids, tannins, terpenes, steroids, and cardiac glycosides. The ethanolic extract displayed the strongest antimicrobial activity against the enteric gram-negative organisms (Proteus sp., K. pneumonia, and E. coli), with an inhibition zone of 14.5 mm. The hot aqueous extract had an inhibition zone

of 12.5 mm against the enteric gram-negative organisms and S. aureus, while the cold aqueous extract had the weakest activity, with an inhibition zone of 11 mm against the enteric bacteria. The minimum inhibitory concentration (MIC) was not determined.

Kolawole et al., (2014) carried out a study to investigate the effect of the leaf extract of *Newbouldia laevis* on some enzymes of hepatic glucose metabolism in induced diabetic rats. Intravenous injection of streptozotoan in the rat was used to induce diabetes and for four weeks the rats were orally treated with *Newbouldia laevis* leaf extract. The activities of hepatic glucokinase and glucose 6-phospate were evaluated at the end of the study and it was deduced that the *Newbouldia laevis* leaf extract stimulates the activity of hepatic glucokinase and inhibits the activity of glucose 6- phosphate in induced diabetic rats.

Usman et al, (2008) did a study on the analgesic and anti-inflammatory effects of the ethanolic flower extract of *Newbouldia laevis* in fifty rodents. They were induced with acetic acid and carrageenan-induced hind paw edema in rats. The results showed that the ethanol extract possessed significant ($p<0.001$) anti-nociceptive activity between 50 and 200 mgkg-' intraperitoneally in mice and also dose dependent anti-inflammatory activity between 50 and 200mgkg' in rats (the extract exhibited highest toxicity at this value).

Fatunla et al. (2016) conducted a study to evaluate the ability of *Newbouldia laevis* leaf extract to inhibit the growth of bacteria. They obtained vancomycin and methicillin-resistant bacterial isolates from urine samples collected at the Federal Medical Center in Owo. These isolates were tested for sensitivity to antibiotics. The researchers prepared methanol extracts of *Newbouldia laevis* using a cold extraction method and tested the extracts for their ability to inhibit the growth of the bacterial isolates using an agar well diffusion technique. The results showed that the methanol extract had strong antibacterial activity against the isolated bacteria.

Although Agbafor et al., (2015) found similar results in the phytochemical analysis of the leaf and root extracts of *Newbouldia laevis*, during their investigation on the cardio-protective effect of the leaf and root extracts of *Newbouldia laevis* against carbon tetrachloride induced-cardiotoxicity in albino rats, the analysis also revealed the presence of Tannins in the leaf extract and Glycosides in the root extract. The presence of these phytochemicals gives the nudge to further investigations on the plant's ability in dealing with specific pathological cases. Investigations seem to be geared towards its effect on inflammatory conditions and infective conditions, usually bacterial and fungal infections.

Nandita and Nana (2001) carried out a study on the antibacterial effect of *Newbouldia laevis* and *Aspilia Africana*. The acetone extracts of both plant parts showed less antibacterial activity compared to the methanol extracts. No significant antibacterial activity was observed in the aqueous extracts. Warming to 60"C significantly increased the sensitivity of the acetone extract of A. africana to the test organisms. At pH 2 and pH 8 the sensitivity of the extracts to the test organisms was same to the non-treated extracts. The MIC (0.60-0.85mg/ml) and MBC (0.70 - 1.02 mg/ml) of the methanol extracts of both plant parts were higher than those of tetracycline and gentamycin (MIC- 0.20- 0.35 and MBC 0.30-0.50).

Chukwujekwu, et al. (2005) did an investigation the antibacterial, anti-inflammatory and antimalarial activity of some Nigerian medicinal plants, in which *Newbouldia laevis* was one of the plant species investigated. Cyclooxygenase (COX-1 and COX-2) assays were used to test for inflammatory activity. All the plant species, except for two, showed anti-inflammatory activity with *Newbouldia laevis* root extract having one of the highest activity (86‡1.9% with petroleum ether root extract, and 781.4% with dichloromethane root extract) of prostaglandin synthesis inhibition, thereby treating inflammation.

Suleiman (2015) conducted a study at Ahmadu Bello University in Zaria to assess the ability of aqueous leaf extract from *Newbouldia laevis* to inhibit the growth of certain bacteria associated with wound infections. In his study, Suleiman (2015) performed preliminary phytochemical analysis and in vitro antibacterial testing on ethanolic leaf extract of *Newbouldia laevis*. The extract was found to contain alkaloids, cardiac glycosides, steroids, and saponins. The antibacterial activity of the extract was evaluated using agar diffusion techniques on clinical isolates of Staphylococcus aureus and Klebsiella pneumonia. The extract did not inhibit the growth of the test organisms at concentration between 250mg/ml to 3.125mg/ml. this study therefore did not justify the traditional use of the plant as remedy for wound healing. He later stated that the resistance by the organism to the aqueous extract may be due to absence of potent secondary metabolites on the leaves which act synergically.

2.2 Effect Of *Newbouldia Laevis* On Intraocular Pressure

Newbouldia laevis leaves extracts have been used for various medicinal purposes, including reducing inflammation, inhibiting the growth of bacteria, and treating malaria. However, their effect on intraocular pressure has not been studied. This research aims to fill this gap by examining the effect of these extracts on intraocular pressure in rabbit models. To the best of our knowledge, this will be the first study to investigate the effect of *Newbouldia lavis* leaves, stem bark and root bark ethanoic extracts on intraocular pressure.

CHAPTER 3

3.0 RESEARCH METHODOLOGY

3.1 RESEARCH DESIGN

This study was an experimental study that was performed on the eyes of 30 live New Zealand rabbits.

3.2 STUDY POPULATION

This study included 30 clinically normal adult New Zealand rabbits, which includes 15 males and 15 females.

3.3 STUDY LOCATION

This study was done in the Animal House of the Department of Animal and Environmental biology, Faculty of Life Science, in the University of Benin, Edo State.

3.4 STUDY DURATION

This study was carried out within a time frame of four (4) weeks.

3.5 SAMPLE SIZE

A total number of thirty (30) subjects was used for this study.

3.6 SAMPLING TECHNIQUE

This study made use of a simple random sampling technique. Simple random sampling, in that 30 rabbits was pooled together and one rabbit was selected at random as a member of a group.

3.7 INCLUSION CRITERIA

- Healthy New Zealand rabbits
- Rabbits within the weight range (2.0kg -3.0kg)
- Rabbits without ocular pathology

3.8 EXCLUSION CRITERIA

- Sick rabbits
- Rabbits with any ocular pathology
- Rabbits outside the stimulated weight range
- Rabbits of different species

3.9 RESEARCH INSTRUMENTS AND MATERIALS

The materials, instruments and drugs used for this research include the following;

- *Newbouldia laevis* leaf, stem and root
- Soxhlet Extractor
- Applanation tonometer
- 30 adult New Zealand rabbits
- Rabbit feed, anticoccidial drugs, antifilarial drugs, multivitamins
- Metal cages
- Weighing balance
- Sterile syringes (2ml and 5ml)
- Topical Anesthetics
- Slit knife
- Dexamethasone tablets B.P. 0.5mg

- Dexamethasone eyedrop 0.1%
- Atropine 1%
- Sterile Fluorescein sodium strips
- Placebo (saline)
- Distilled Water
- Sterile Filter paper
- Beakers, Petri dish, conical flasks
- Sterile drop containers
- Cotton wool
- Methylated Spirit
- Sterile Latex gloves
- Handheld magnifier (8.00-10.00D)
- Penlight
- Direct Ophthalmoscope
- iPhone 12 Pro Camera
- Refrigerator
- Writing materials for documentation
- Blue filter button lamp

3.10 DESCRIPTION OF PROCEDURE

3.10.1 COLLECTION OF LEAF, STEM BARK, AND ROOT BARK OF *NEWBOULDIA LAEVIS* PLANT AND PREPARATION OF THEIR EXTRACT

Fresh leaf, stem bark, and root bark of *Newbouldia laevis* plants was obtained from a garden in the Senior Staff Quarters in the University of Benin. After which they were taken to the Department of Plant Biology and Biotechnology, in the Faculty of Life Sciences for appropriate identification. After which they were washed, the stem and root bark will be cut into pieces and air dried for fourteen days before being grounded into powder using a milling machine. Ethanolic Extraction of the leaf, stem bark and root bark will be carried out in the Department of Pharmacognosy, Faculty of Pharmacy in the University of Benin. Two kilograms of the grinded powder was subjected to Soxhlet extraction with methanol.

3.10.2 ANIMALS AND HANDLING

This study was a controlled experimental study which was divided in two parts: the Adaptive Phase (Phase 1) where the animals was made to adapt to their environment for a period of two week and then the Experimental Phase (Phase 2&3) during which the conditions of the experiment was administered and the treatments was initiated. 30 healthy adult New Zealand rabbits of either sex, with an average weight ranging from 2.0kg -3.0kg, was obtained from an animal farm in Benin City. The Rabbits was kept in metal cages in the Animal House in the Department of Animal and Environmental biology and was given two weeks to adapt to their new environment (acclimatization), with appropriate antibacterial (Keproceryl) and antifiliarial (Ivermectin) and anticoccidial (Embazin) treatments that was administered in the rabbit feed along with multivitamins and nutritional rations .The end of the acclimatization phase (Phase 1) begins the experimental phase (Phase 2) in which the inflammation was inflicted on the rabbits

eyes and also the IOP was increased with the administration of dexamethasone eyedrops and tablet , once the pressure was well increased and the inflammation has set in, the various plants extracts was administered three times daily and the rate of healing of the rabbits was divided into three groups .

3.10.3 Grouping and Handling of Animals

Six rabbits were separated as control for the test. This control group was tagged group G. The remaining 30 rabbits were grouped into seven groups, Groups A, B, C, D, E, F and G. One group was tested for the effect of the extract on one ocular condition. Each group had the respective conditions of either Inflammation or intra ocular pressure induced in their eyes.

GROUP A: This group comprise of four Rabbits that was inflicted with inflammation on their right eyes and this inflammation was attained by the use of ophthalmic slit knife to physically induce injury on the corneo-conjuctival of the rabbits. After twenty-four hours, excess tearing along with other classical signs of uveitis (red eye/ciliary injection, photophobia). (Villena eya love 1999). Baseline data of their Schirmer test was taken and also was taken daily after inflammation was induced. After which they were treated with the ethanolic leaf extracts of *Newbouldia laevis* plant three times daily. Until the inflammation was healed and the Schirmer test value returned to baseline data.

GROUP B: This group comprise of four Rabbits that was inflicted with inflammation on their right eyes and this inflammation was attained by the use of ophthalmic slit knife to physically induce injury on the corneo-conjuctival of the rabbits. After twenty-four hours, excess tearing along with other classical signs of uveitis (red eye/ciliary injection, photophobia). (Villena eya love 1999). Baseline data of their Schirmer test was taken and also was taken daily after

inflammation was induced. After which they were treated with the ethanolic Stem bark extracts of *Newbouldia laevis* plant three times daily. Until the inflammation was healed and the strimmer test value returned to baseline data.

GROUP C: This group comprise of four Rabbits that was inflicted with inflammation on their right eyes and this inflammation was attained by the use of ophthalmic slit knife to physically induce injury on the corneo-conjuctival of the rabbits. After twenty-four hours, excess tearing along with other classical signs of uveitis (red eye/ciliary injection, photophobia). (Villena eya love 1999). Baseline data of their Schirmer test was taken and also was taken daily after inflammation was induced. After which they were treated with the ethanolic root bark extracts of *Newbouldia laevis* plant three times daily.

GROUP D: Group D were tested on induced increase IOP, baseline data of the rabbits' intraocular pressure were collected using the Perkins Tonometer. The rabbits were given one tablet of Dexamethasone orally and had one drop of Dexamethasone 0.1%, then one drop Atropine 1% instilled in one eye (right eye) four times daily for two weeks. After 2 weeks the steroid had artificially increased the intraocular pressure of the rabbits' eyes. After which they were treated with the ethanolic Stem bark extracts of *Newbouldia laevis* plant three times daily

GROUP E: This group of rabbit were tested on induced increase IOP, baseline data of the rabbits' intraocular pressure were collected using the Perkins Tonometer. The rabbits were given one tablet of Dexamethasone orally and had one drop of Dexamethasone 0.1%, then one drop Atropine 1% instilled in one eye (right eye) four times daily for two weeks. After 2 weeks the steroid had artificially increased the intraocular pressure of the rabbits' eyes. After which they were treated with the ethanolic root bark extracts of *Newbouldia laevis* plant three times daily

GROUP F: This group of rabbit were tested on induced increase IOP, baseline data of the rabbits' intraocular pressure were collected using the Perkins Tonometer. The rabbits were given one tablet of Dexamethasone orally and had one drop of Dexamethasone 0.1%, then one drop Atropine 1% instilled in one eye (right eye) four times daily for two weeks. After 2 weeks the steroid had artificially increased the intraocular pressure of the rabbits' eyes. After which they were treated with the ethanolic leaf bark extracts of *Newbouldia laevis* plant three times daily.

GROUP G : This group will serve as the control. This group of animals won't have any kind of physically caused eye inflammation or elevated intraocular pressure. Additionally, no ethanolic extracts of the *Newbouldia laevis* plant may be given to them. There will be six rabbits in it

3.11 LIMITATIONS OF STUDY

- The cost to carry out the study might be very high due to the current state of recession in the country
- It can really take a long time for meaningful results to be gotten.
- The number of the test subjects might be reduced due to unexpected death thereby causing a reduction in sample size
- The laboratory environment and other variables might have an effect on study outcomes.

3.12 DATA ANALYSIS

Data collected was analyzed using analysis of variance (ANOVA) as processed by Statistical Package for social sciences (version 22.0). IOP data was expressed as the mean and was analyzed by a two-tailed paired t-test for ratios compared to 1.0 or for differences compared with 0.0. The grading was done using the Vision Care Institute Clinical grading scale for detection of changes in the ocular inflammation, which is as follows:

0- Normal

1- Trace defects

2- Mild

3- Moderate defects present

4- Severe defects present

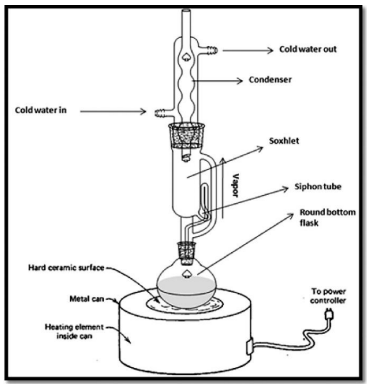

Figure 3.1: Soxhlet extractor adapted from Dasari, Swaroopa & Goud, Vaibhav. (2014).

Figure 3.2 : Freshy picked *Newbouldia laevis* leaf

CHAPTER FOUR

4.0 Result Presentation

A total of thirty (no = 30) rabbits was used for this study. The rabbit was divided into 3 major groups, 12 rabbits in two group and 6 rabbits as the control group. One group was for IOP while the other group was for Inflammation.

In IOP group of the 12 rabbits, it was further divided into 3 groups (Group A, Group B and Group C). In Group A, IOP was induced and treated with Leaf extract, in Group B, IOP was induced and treated with Stem extract and in the Group C, IOP was induced and treated with Root extract.

Also, for the other group of 12 rabbits was induced for inflammation, it was divided into Group A, Group B and Group C in which inflammation was induced and each groups treated using Leaf extract, Stem extract and Root extract, respectively.

Table 4.2: Mean values and 95% Confidence Interval for Rabbits induced for Corneo-Conjuctival Inflammation

Table 4.1: Mean values and 95% Confidence Interval for Rabbits induced for increased IOP

Extract	Days	Mean ± SD	95% Confidence Interval
Group A (Leaf)	Baseline IOP	9.75 ± 1.26	9.75 ± 1.23
	IOP after inducing for 7 days	19.50 ± 1.29	19.50 ± 1.27
	IOP after 3 days of Treatment	14.50 ± 1.29	14.50 ± 1.27
	IOP after 5 days of Treatment	11.25 ± 0.96	11.25 ± 0.94
Group B (Stem)	Baseline IOP	13.50 ± 2.87	13.50 ± 2.82
	IOP after inducing for 7 days	21.00 ± 2.58	21.00 ± 2.53
	IOP after 3 days of Treatment	17.00 ± 1.16	17.00 ± 1.13
	IOP after 5 days of Treatment	12.75 ± 2.87	12.75 ± 2.82
Group C (Root)	Baseline IOP	10.75 ± 1.89	10.75 ± 1.86
	IOP after inducing for 7 days	20.50 ± 2.08	20.50 ± 2.04
	IOP after 3 days of Treatment	16.00 ± 2.58	16.00 ± 2.53
	IOP after 5 days of Treatment	12.75 ± 2.22	12.75 ± 2.17

This table shows the mean value and standard deviation of variables as we as 95% Confidence Interval for increased IOP

Extract

Table 4.3: Compared the Efficacy of Ethanolic Extracts of The Leaf, Stem and Root of

Group A	TFR after inducing for 7 days	14.25 ± 1.71	14.25 ± 1.67
	TFR after 1 days of Treatment	14.25 ± 1.71	14.25 ± 1.67
	TFR after 2 days of Treatment	8.75 ± 2.22	8.75 ± 2.17
	TFR after 3 days of Treatment	7.00 ± 2.45	7.00 ± 2.40
	TFR after 4 days of Treatment	12.75 ± 2.87	12.75 ± 2.81
	TFR after 5 days of Treatment	9.25 ± 2.22	9.25 ± 2.17
Group B (Stem)	Baseline TFR	13.75 ± 2.06	13.75 ± 2.03
	TFR after inducing for 7 days	18.25 ± 2.50	18.25 ± 2.45
	TFR after 1 days of Treatment	18.25 ± 2.50	18.25 ± 2.45
	TFR after 2 days of Treatment	14.50 ± 1.73	14.50 ± 1.70
	TFR after 3 days of Treatment	12.00 ± 1.63	12.00 ± 1.60
	TFR after 4 days of Treatment	11.25 ± 1.50	11.25 ± 1.47
	TFR after 5 days of Treatment	9.75 ± 0.50	9.75 ± 0.49
Group C (Root)	Baseline TFR	12.00 ± 3.56	12.00 ± 3.49
	TFR after inducing for 7 days	16.00 ± 4.69	16.00 ± 4.60
	TFR after 1 days of Treatment	16.00 ± 4.69	16.00 ± 4.60
	TFR after 2 days of Treatment	14.75 ± 4.57	14.75 ± 4.48
	TFR after 3 days of Treatment	10.25 ± 4.11	10.25 ± 4.03
	TFR after 4 days of Treatment	15.25 ± 3.30	15.25 ± 3.24
	TFR after 5 days of Treatment	12.25 ± 3.30	12.25 ± 3.24

This table shows the mean value and standard deviation of variables as we as 95% Confidence Interval for inflammation

***Newbouldia Laevis* on Increased Intraocular Pressure**

		df	F	p-values
Baseline of IOP	Between Groups	2	2.907	.106
	Within Groups	9		
	Total	11		
7 days after inducing increased IOP	Between Groups	2	.553	.594
	Within Groups	9		
	Total	11		
IOP of Day 3 Treatment	Between Groups	2	1.966	.196
	Within Groups	9		
	Total	11		
IOP of Day 5 Treatment	Between Groups	2	.639	.550
	Within Groups	9		
	Total	11		

This table shows the efficacy of the various extract of for increased IOP.

Table 4.4: Compared the Efficacy of Ethanolic Extracts of The Leaf, Stem and Root of

***Newbouldia Laevis* on Corneo-Conjuctival Inflammation**

		df	F	p-values
Baseline of TRF	Between Groups	2	2.946	.104
	Within Groups	9		
	Total	11		
TFR after inducing Corneo-	Between Groups	2	1.548	.264
Conjuctival inflammation	Within Groups	9		
	Total	11		
TFR of Day 3 Treatment	Between Groups	2	3.020	.099
	Within Groups	9		
	Total	11		
TFR of Day 5 Treatment	Between Groups	2	1.927	.201
	Within Groups	9		
	Total	11		

This table shows the efficacy of the various extract of for inflammation.

ANOVA

IOP Group B Stem

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	176.500	3	58.833	9.605	.002
Within Groups	73.500	12	6.125		
Total	250.000	15			

IOP: Intra ocular pressure

The ANOVA comparison between groups A (leaf) and within groups was compared and there was significance difference between group, a P value less than 0.05 was taken to be statistically significant.

IOP: Intra ocular pressure

The ANOVA comparison between groups C (root) and within groups was compared and there was significance difference between group, a P value less than 0.05 was taken to be statistically significant.

.

IOP: Intra ocular pressure

ANOVA

TFR_Group_A_Leaf

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	111.500	3	37.167	9.591	.002
Within Groups	46.500	12	3.875		
Total	158.000	15			
[illegible]	217.500	3	72.500	14.872	.001
Within Groups	58.500	12	4.875		
Total	276.000	15			

The ANOVA comparison between groups C (root) and within groups was compared and there was significance difference between group, a P value less than 0.05 was taken to be statistically significant.

TFR: Tear flow rate

The ANOVA comparison between groups and within groups was compared and there was significance difference between group, a P value less than 0.05 was taken to be statistically significant.

ANOVA

TFR_Group_B_Stem

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	155.688	3	51.896	15.472	.001
Within Groups	40.250	12	3.354		
Total	195.938	15			

TFR: Tear flow rate

The ANOVA comparison between groups and within groups was compared and there was significance difference between group, a P value less than 0.05 was taken to be statistically significant.

ANOVA

TFR_Group_C_Root

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	70.250	3	23.417	1.499	.265
Within Groups	187.500	12	15.625		
Total	257.750	15			

TFR: Tear flow rate

The ANOVA comparison between groups and within groups was compared and there was no significance difference. A P value less than 0.05 was taken to be statistically significant.

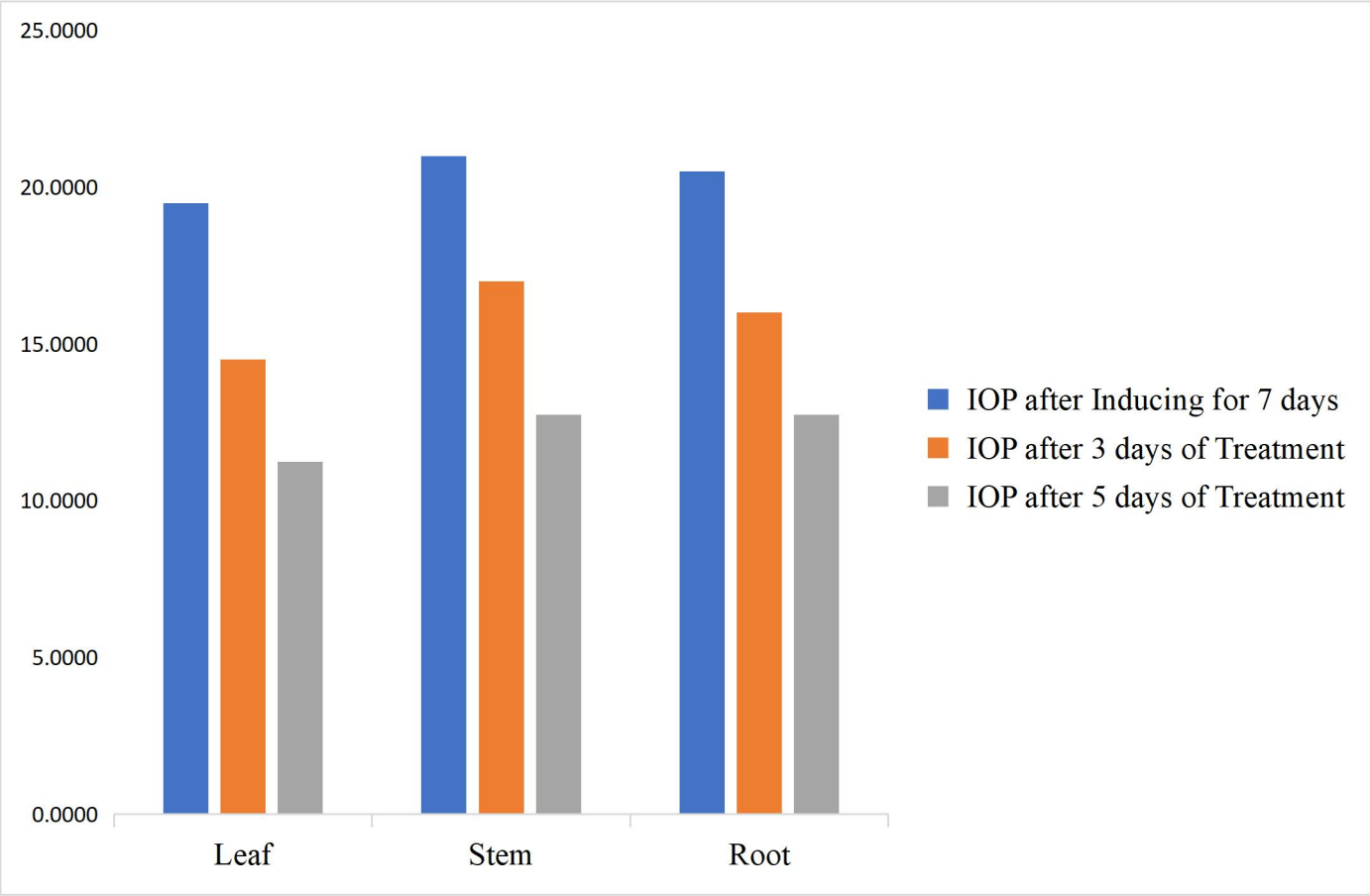

Figure 4.1: Bar chart showing Mean values of ethanolic extract Leaf, Stem and Root on increased IOP

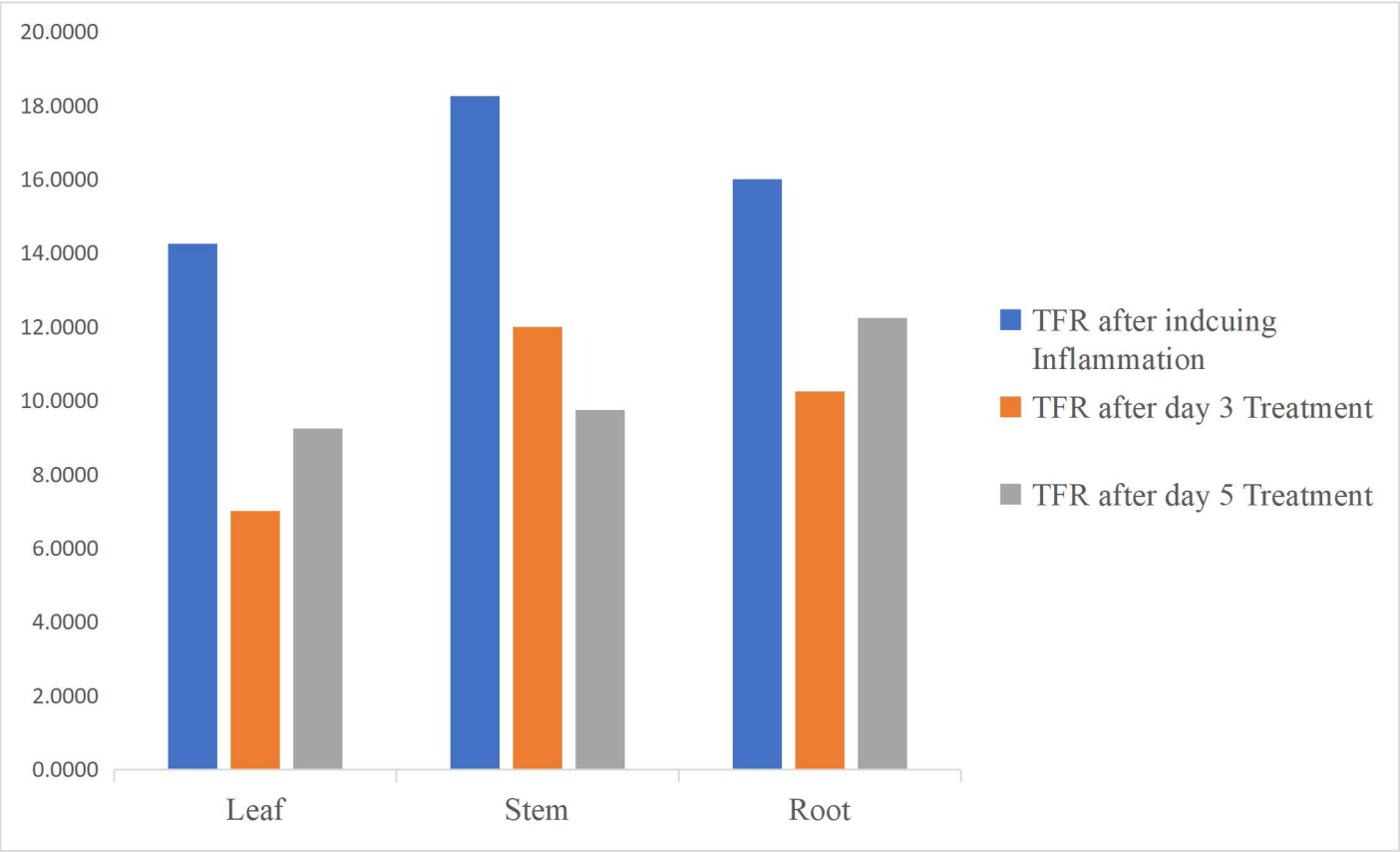

Figure 4.2: Bar chart showing Mean values of TFR after administration of ethanolic extract (Leaf, Stem and Root) on induced Corneo-Conjuctival inflammation

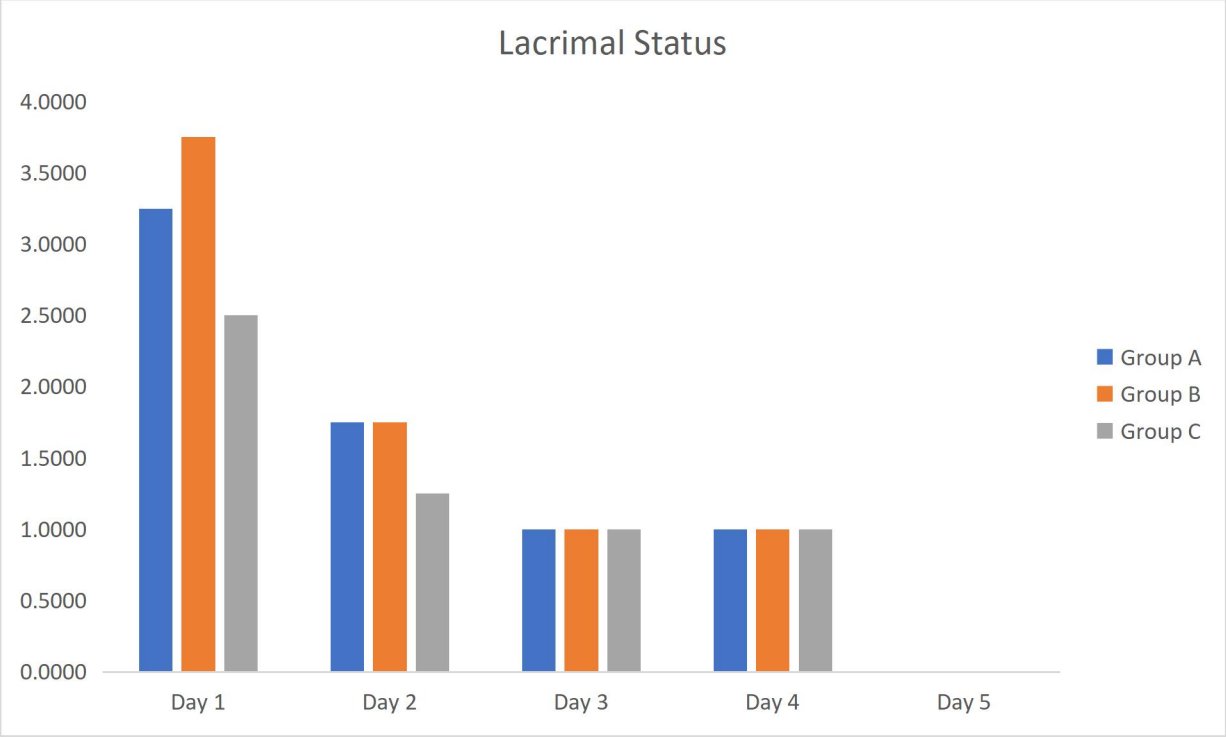

Figure 4.3: Bar chart showing Mean values of Lacrimal Status of the eyes after administration of ethanolic extract (Leaf, Stem and Root) on induced Corneo-Conjuctival inflammation

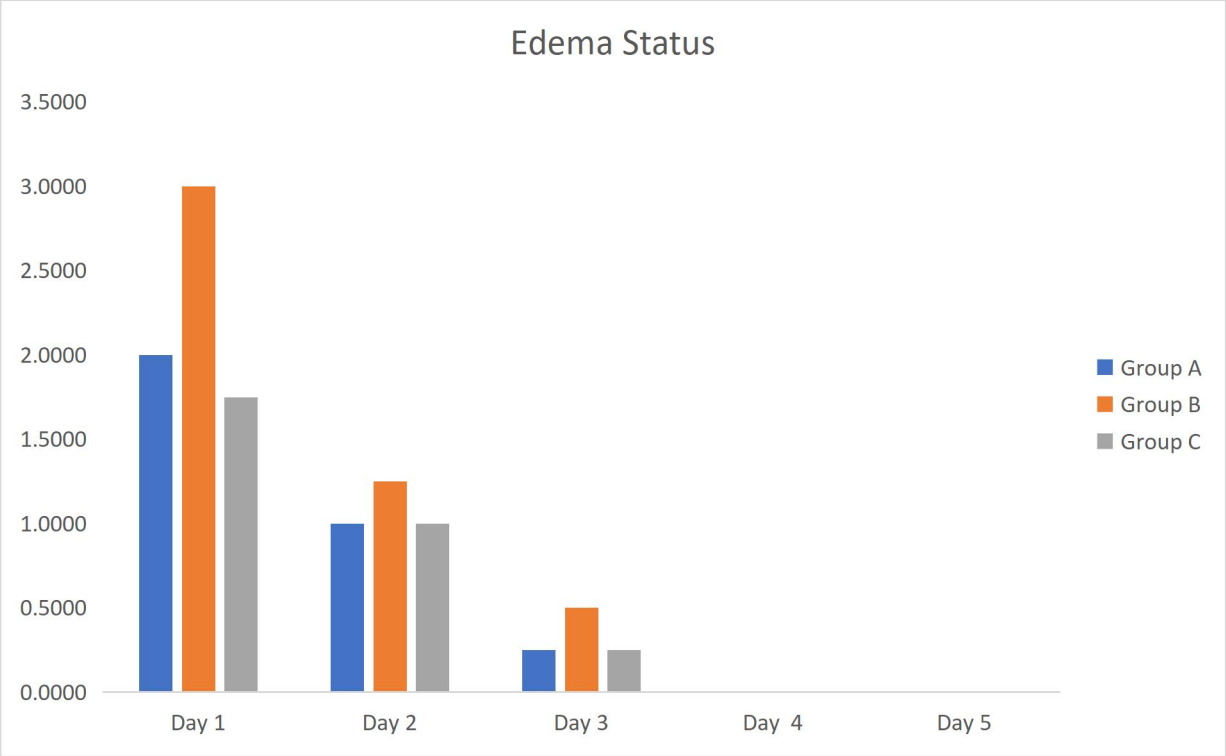

Figure 4.4: Bar chart showing Mean values of Edema Status of the eyes after administration of ethanolic extract (Leaf, Stem and Root) on induced Corneo-Conjuctival inflammation

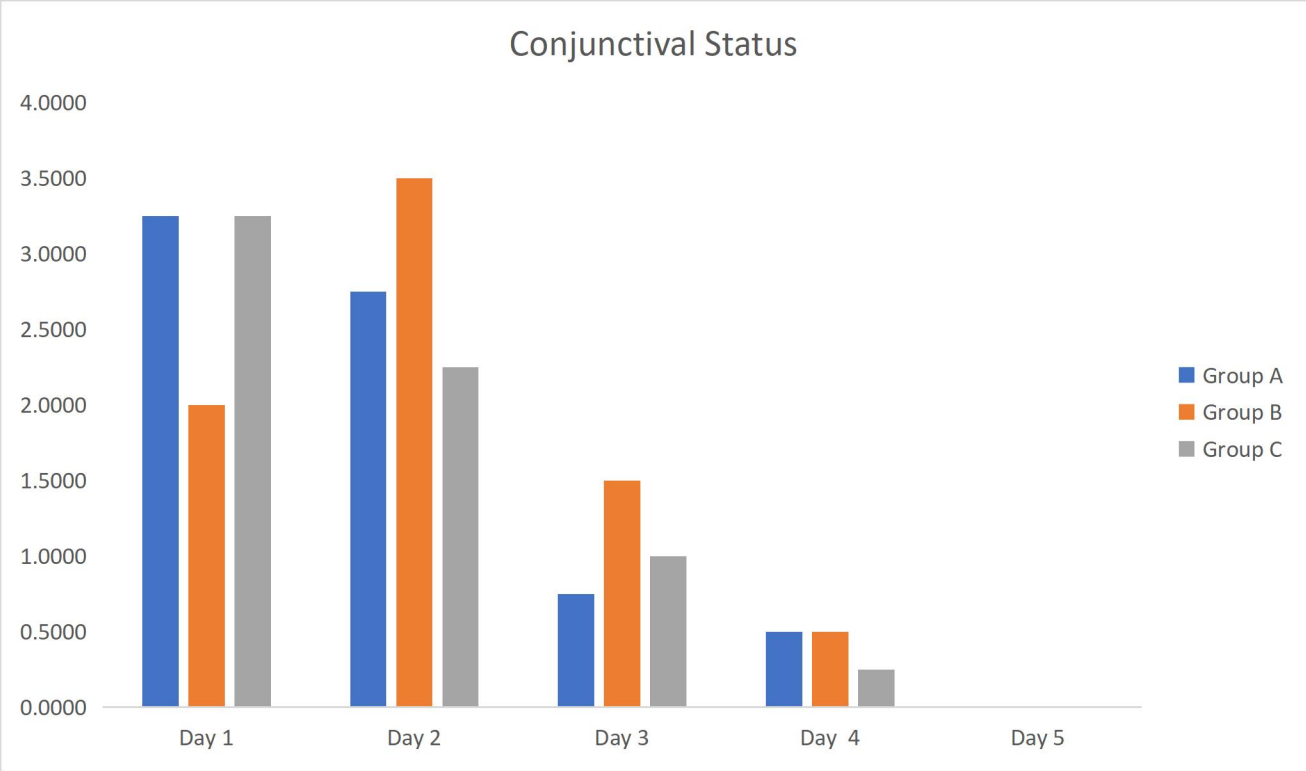

Figure 4.5: Bar chart showing Mean values of conjunctiva Status of the eyes after administration of ethanolic extract (Leaf, Stem and Root) on induced Corneo-Conjuctival inflammation

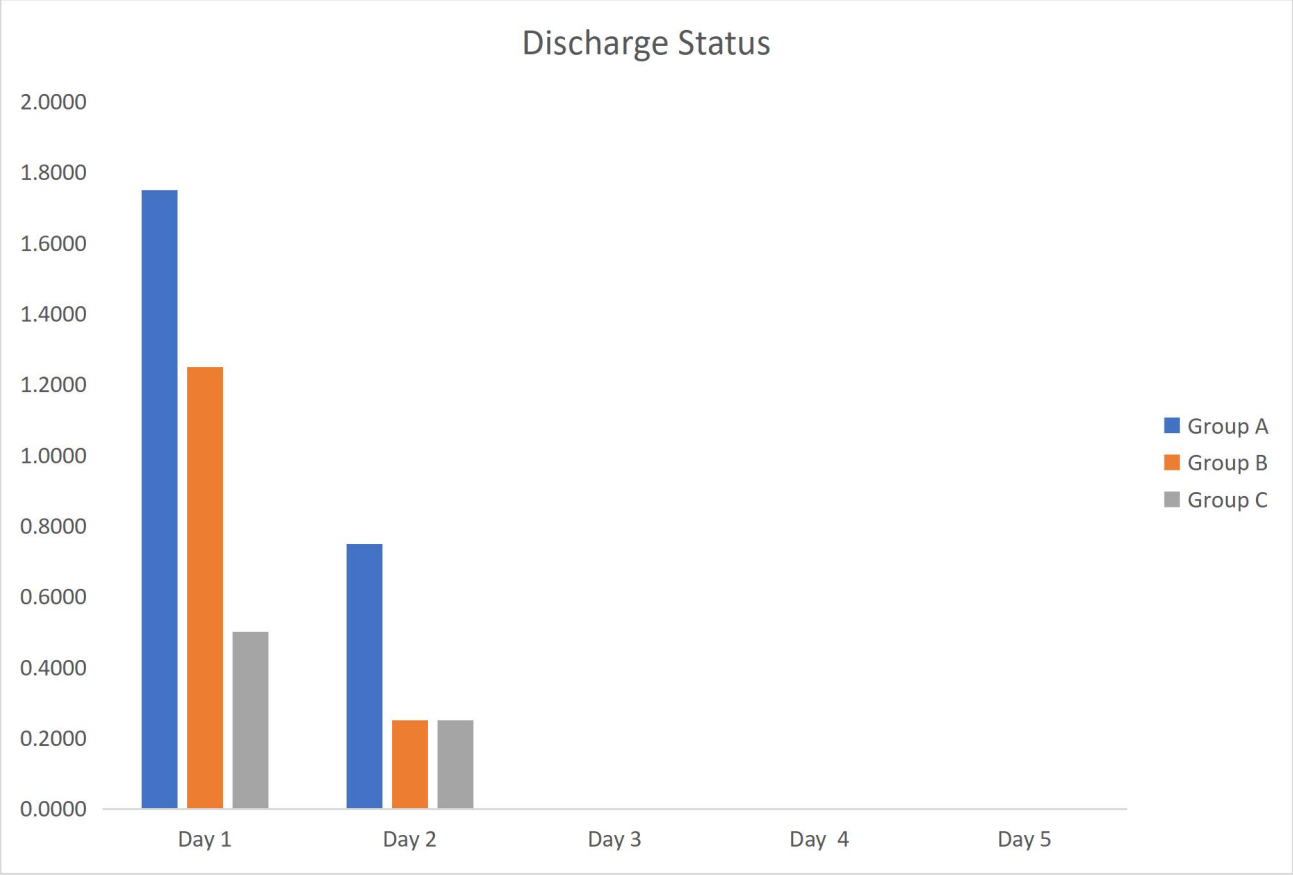

Figure 4.6: Bar chart showing Mean values of Discharge Status of the eyes after administration of ethanolic extract (Leaf, Stem and Root) on induced Corneo-Conjuctival inflammation

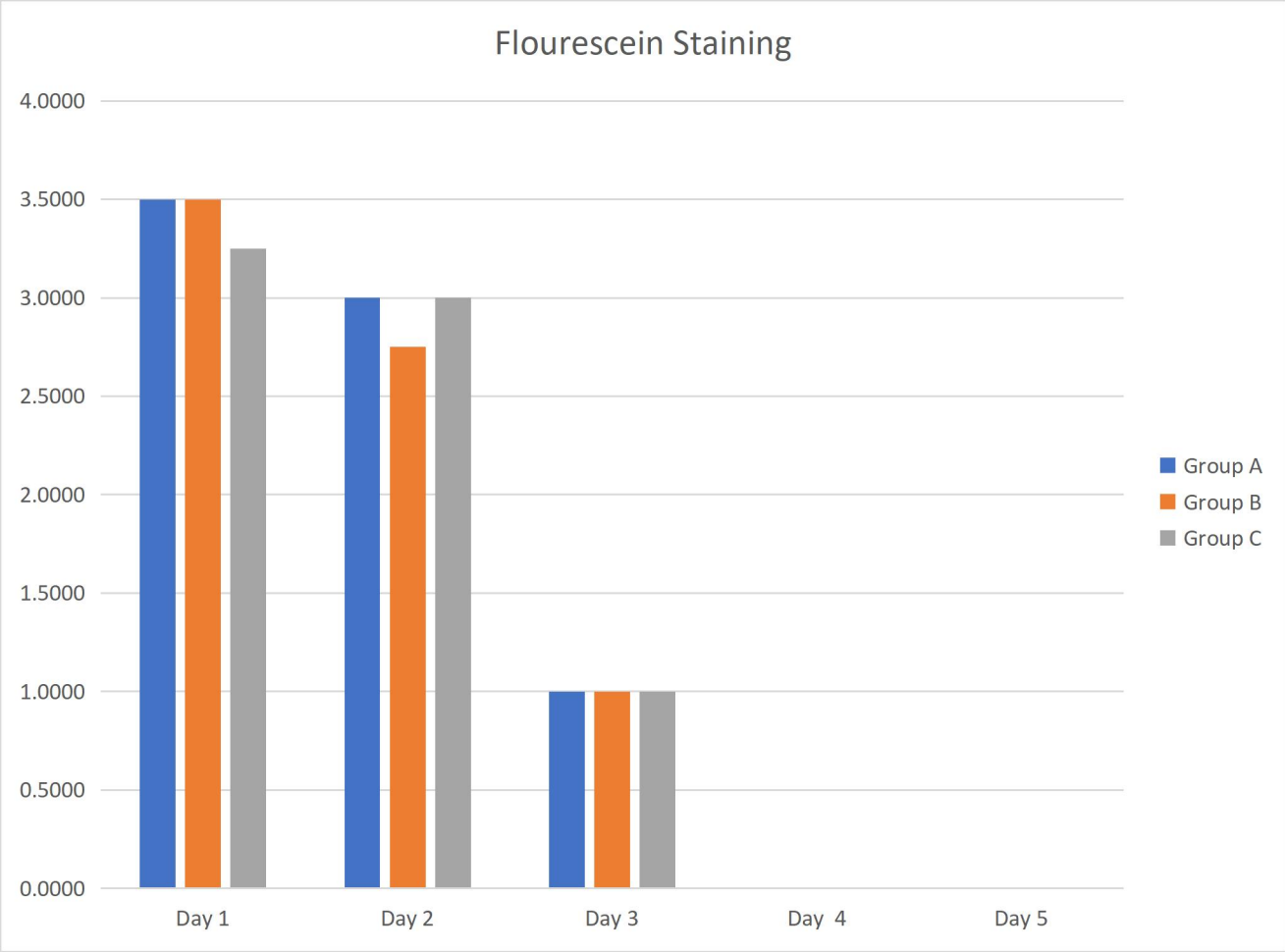

Figure 4.7: Bar chart showing Mean values of Fluorescein Staining Status of the eyes after administration of ethanolic extract (Leaf, Stem and Root) on induced Corneo-Conjuctival inflammation

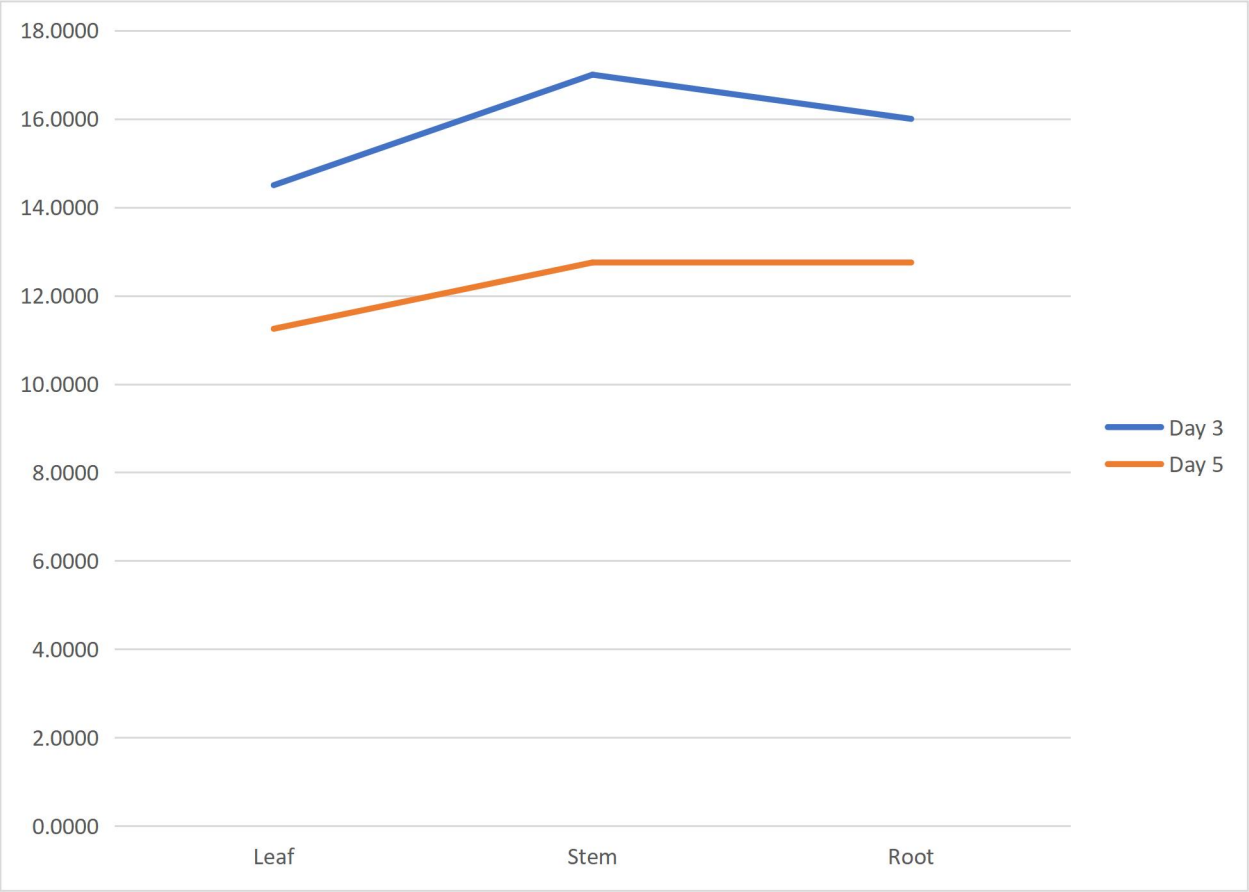

Figure 4.8: Line chart showing the Healing Progress of the three (3) Extracts on increased IOP

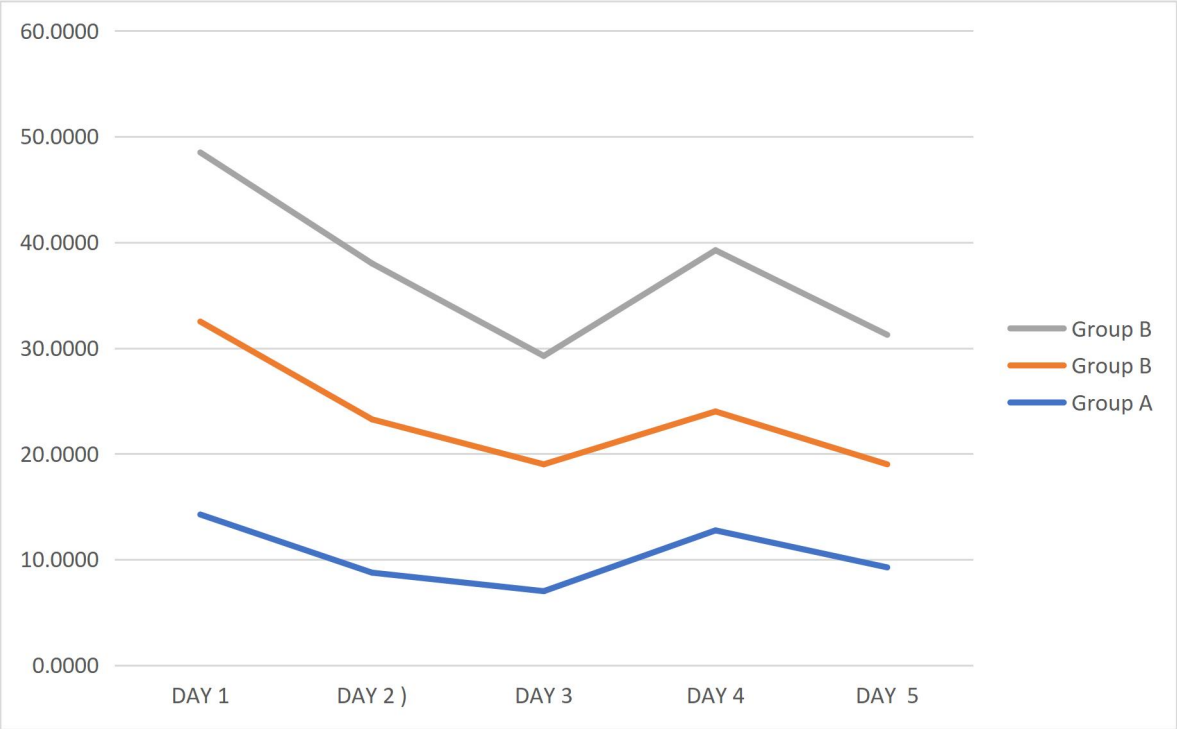

Figure4.9: Line chart Healing Progress of the three (3) Extracts on induced inflammation

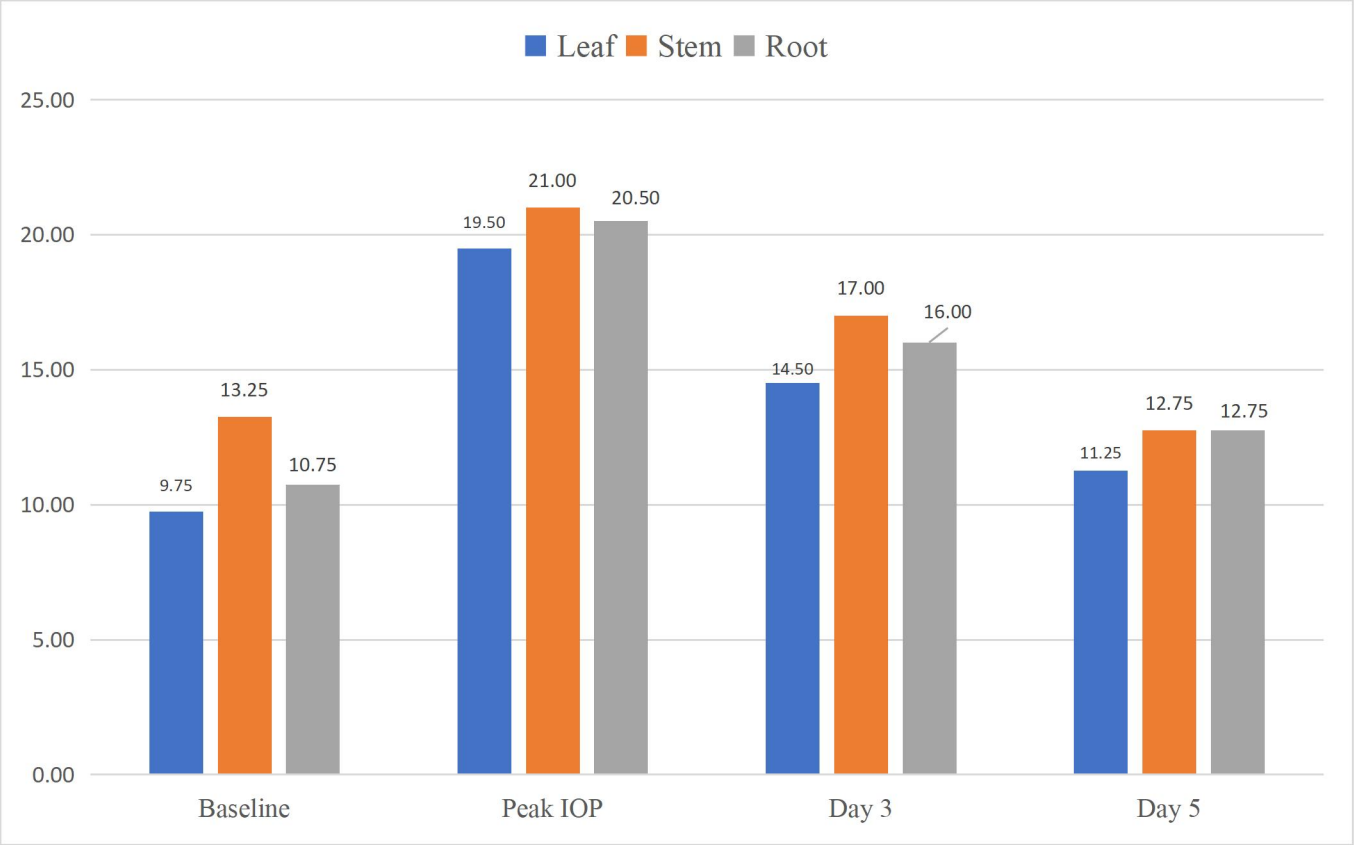

Figure 4.10: Bar chart showing Mean values of ethanolic extract Leaf, Stem and Root on increased IOP at different days

figure 4.10: measurement of tear flow rate, using Schirmer test

figure 4.11: inducement of IOP with dexamethasone eyedrop

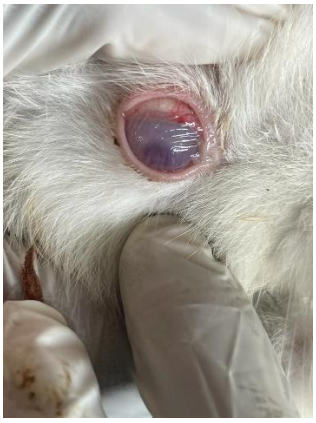

figure 4.12: inflamed eye

figure 4.13: lacerating the rabbits eye with a slit knife

CHAPTER FIVE

5.0 DISCUSSION

Despite the wide range of clinical use of *Newbouldia laevis* plant from aiding in uterine contraction, stomach ache healing, aid to heal toothache, treating constipation, pile, syphilis, and dysentery, and its anti-inflammatory activity, no much clinical work has been done on the effect *Newbouldia laevis* on elevated intra ocular pressure.

In this study, the effects of the leaf, stem and root bark extract of *Newbouldia laevis* on corneo-conjuctiva inflammation and intra ocular pressure were determined.

After 24 hours of causing laceration on the corneas of the rabbits using a slit knife in the recipient groups viz - groups A, B and C, it was observed that marked inflammatory responses: edema, conjunctival injection, lacrimation and mucus discharge, were present.

The anti-inflammatory effect of *Newbouldia laevis* leaf, stem and root bark extract the eyes of the rabbits in groups A,B,C was observed over a period of 5 days and their inflammatory status was observed to be healed by the fourth day of treatment. In study carried out by Lui et al (2020), on the IOP reducing effect of Bilberry *(Vaccinium myrtillus),* it was found that the active component anthocyanin, which is a flavonoid, is also found in *Newbouldia laevis* helped in the reduction of IOP. My research study is in comparison with the study.

When One-Way ANOVA was used to determine the effects of leaf extract on Group A rabbit before, after inducing, 3 days after treatment and 7 days after treatment of IOP, it was found to be statistically significant ($F = 51.09$; $df = 3, 12$; $p = 0.000$). there was also significant difference before, after inducing, 3 days after treatment and 7 days after treatment of IOP when stem and root extract were used, (One-Way ANOVA: $F = 9.61$; $df = 3, 13$; $p = 0.002$) and (One-Way ANOVA: $F = 14.87$; $df = 3, 13$; $p = 0.000$), respectively. Most of the post-hoc test results shows

that there was significant difference before, after inducing, 3 days after treatment and 7 days after treatment of IOP in the extracts.

Furthermore, One-Way ANOVA was used to determine the effect of the leaf, stem and root extract on the tear flow rate (TFR) of three different groups of rabbits before, after inducing, 3 days after treatment and 7 days after treatment of inflammation, there was statistically significant difference in the leaf and stem, ($F = 9.59$; $df = 3, 12$; $p = 0.002$) and ($F = 15$; df; 3, 12; $p = 0.000$), respectively, but the root shows no statistically significant difference in the TFR before, after inducing, 3 days after treatment and 7 days after treatment of IOP ($F = 1.50$; $df = 3, 12$; $p = 0.265$).

When the efficacy of ethanolic extracts of the leaf, stem and root of *Newbouldia laevis* on increased intraocular pressure was compared using One-Way ANOVA, the results shows that there was no statistically significant difference between the three (3) ethanolic extracts (leaf, stem and root) (Table 4.3).

Also, when the efficacy of ethanolic extracts of the leaf, stem and root of *Newbouldia laevis* on corneo-conjuctival inflammation was compared using One-Way ANOVA, the results shows that there was no statistically significant difference between the three (3) ethanolic extracts (leaf, stem and root) (Table 4.4).

CHAPTER SIX

6.0 Conclusion and Recommendations

6.1 Conclusion

From this research carried out, it can be concluded that the leaf, stem bark and root extract of *Newbouldia laevis* plant was seen to have an effect in treating the two induced ocular conditions (inflammation and increased intra ocular pressure. The study revealed a positive effect of the use of this extract on the stated conditions.

This further shows that the leaf, stem bark and root extract of *Newbouldia laevis* plant can be used to reduce intra ocular pressure and to manage conjunctival inflammation.

6.2 Recommendations

Following the results obtained from this study, we suggest that

- Awareness on the anti-inflammatory and intra ocular pressure reducing effect of leaf, stem bark and root extract of *Newbouldia laevis* plant. This is not to say that we recommend direct application of the extract on the eyes, as other factors such as chemical purity and the right dosage of the active components of these substances are paramount (proper standardization by the relevant authorities of this substance is necessary).
- In depth analysis of these extracts should be carried out to show the active ingredients
- From the findings of the study, we suggest that medicinal plant like *Newbouldia laevis* should not be totally neglected by clinicians for treatment of ocular conditions like inflammation and increased IOP.

- Further studies should be carried out using humans diagnosed with corneo – conjunctival inflammation and increased IOP experimental subjects and the number of days of observation increased to at least 10 days with daily application of the treatment
- Further studies should be conducted to see if this extract can be turned into drugs

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APPENDIX

IOP DATA

GROUP	ANIMAL	BASELINE IOP (mmhg)	PEAK IOP AFTER INDUCING (mmhg)	IOP AFTER INDUCING FOR 1 DAY (mmhg)	IOP AFTER INDUCING FOR 3 DAYS (mmhg)	IOP AFTER 5 DAYS OF TREATMENT (mmhg)	IOP AFTER 7 DAYS OF TREATMENT (mmhg)
A(LEAF)	R_1	8	21	20	18	13	10
	R_2	10	23	23	20	14	12
	R_3	10	23	22	19	16	12
	R_4	11	21	21	18	15	11
B(STEM)	R_5	15	20	18	16	13	9
	R_6	15	20	21	18	16	12
	R_7	14	22	22	20	18	15
	R_8	9	20	20	18	16	15
C (ROOT)	R_9	12	21	19	16	13	10
	R_{10}	11	23	21	19	15	12
	R_{11}	8	21	21	19	17	14
	R_{12}	12	22	22	20	18	15

IOP CONTROL DATA

CONTROL		BASELINE (mmhg)	AFTER TREATMENT (mmhg)
	R_1	9	11
	R_2	16	14
	R_3	12	10

TEAR FLOW RATE

GROUP	ANIMAL	BASELINE (mm)	AFTER INFLAMMATION (mm)	DAY 1 OF TREATMENT (mm)	DAY 2 OF TREATMENT (mm)	DAY 3 OF TREATMENT (mm)	DAY 4 OF TREATMENT (mm)	DAY 5 OF TREATMENT (mm)
A (LEAF)	R_1	10	14	14	10	5	13	8
	R_2	11	15	15	8	10	16	12
	R_3	9	16	16	11	8	13	10
	R_4	8	12	12	6	5	9	7
B (STEM)	R_5	12	19	19	15	12	12	10
	R_6	12	15	15	12	10	9	9
	R_7	15	21	21	16	12	12	10
	R_8	16	18	18	15	14	12	10
C (ROOT)	R_9	9	10	10	9	5	11	8
	R_{10}	10	18	18	16	11	16	12
	R_{11}	12	15	15	14	10	15	13
	R_{12}	17	21	21	20	15	19	16

INFLAMMATION DATA

DAY 1

GROUP	LACRIMATION	EYELID STATUS	EDEMA	CONJUCTIVAL STATUS	DISCHARGE	FLOURESCEIN STAINING
A (LEAF) R_1	3	0	2	4	2	4
R_2	3	0	2	3	3	4
R_3	4	0	3	3	1	3
R_4	3	0	1	3	1	3
B (STEM) R_5	4	0	2	4	2	4
R_6	3	0	4	4	2	3
R_7	4	0	3	3	1	4
R_8	4	0	3	3	0	3
C (ROOT) R_9	3	0	2	3	0	4
R_{10}	2	0	2	4	2	3
R_{11}	2	0	2	3	0	3
R_{12}	3	0	1	3	0	3

DAY 2

GROUP	ANIMAL	LACRIMATION	EYELID STATUS	EDEMA	CONJUCTIVAL STATUS	DISCHARGE	FLOURESCEIN STAINING
A(LEAF)	R_1	2	0	2	3	1	4
	R_2	2	0	1	3	1	3
	R_3	1	0	0	2	0	2
	R_4	2	0	1	3	1	3
B(STEM)	R_5	2	0	0	2	0	2
	R_6	2	0	2	2	1	3
	R_7	1	0	2	3	0	4
	R_8	2	0	1	1	0	2
C(ROOT)	R_9	1	0	1	3	0	4
	R_{10}	1	0	2	2	1	3
	R_{11}	1	0	1	2	0	3
	R_{12}	2	0	0	2	0	2

DAY 3

GROUP	ANIMAL	LACRIMATION	EYELID STATUS	EDEMA	CONJUCTIVAL STATUS	DISCHARGE	FLOURESCEIN STAINING
A(LEAF)	R_1	1	0	1	2	0	2
	R_2	1	0	0	1	0	1
	R_3	1	0	0	0	0	1
	R_4	1	0	0	0	0	0
B(STEM)	R_5	1	0	0	1	0	1
	R_6	1	0	1	2	0	1
	R_7	1	0	1	2	0	2
	R_8	1	0	0	1	0	0
C(ROOT)	R_9	1	0	0	2	0	2
	R_{10}	1	0	1	2	0	1
	R_{11}	1	0	0	0	0	0
	R_{12}	1	0	0	0	0	1

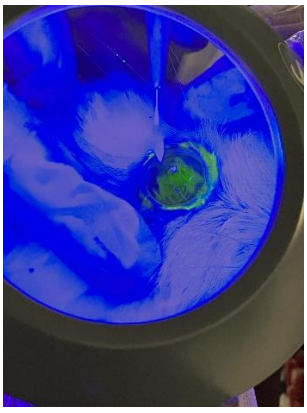
DAY 4

GROUP	ANIMALS	LACRIMATION	EYELID STATUS	EDEMA	CONJUCTIVAL STATUS	DISCHARGE	FLOURESCEIN STAINING
A(LEAF)	R_1	1	0	0	1	0	0
	R_2	1	0	0	1	0	0
	R_3	1	0	0	0	0	0
	R_4	1	0	0	0	0	0
B(STEM)	R_5	1	0	0	1	0	0
	R_6	1	0	0	0	0	0
	R_7	1	0	0	1	0	0
	R_8	1	0	0	0	0	0
	R_9	1	0	0	0	0	0
	R_{10}	1	0	0	1	0	0
	R_{11}	1	0	0	0	0	0
	R_{12}	1	0	0	0	0	0

DAY 5

GROUP	ANIMAL	LACRIMATION	EYELID STATUS	EDEMA	CONJUCTIVAL STATUS	DISCHARGE	FLOURESCEIN STAINING
A(LEAF)	R_1	0	0	0	0	0	0
	R_2	0	0	0	0	0	0
	R_3	0	0	0	0	0	0
	R_4	0	0	0	0	0	0
B(STEM)	R_5	0	0	0	0	0	0
	R_6	0	0	0	0	0	0
	R_7	0	0	0	0	0	0
	R_8	0	0	0	0	0	0
C(ROOT)	R_9	0	0	0	0	0	0
	R_{10}	0	0	0	0	0	0
	R_{11}	0	0	0	0	0	0
	R_{12}	0	0	0	0	0	0

Oral application of systemic dexamethasone



Fluorescein stained, lacerated cornea

Edema of the palpebral conjunctival of the inflamed eye