# NEUROPROTECTIVE POTENTIAL OF *LANTANA trifolium* ETHANOLIC EXTRACT AGAINST ETHAMBUTOL INDUCED CHANGES IN THE OPTIC NERVE

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## DECLARATION

I, OWEMBABAZI ELNA, declare that this research dissertation is my original work and has never been submitted in any form for any other award.

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## SUPERVISORS APPROVAL

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## DEDICATION

This work is dedicated to my late grandparents Mr. Sabiti Peter and Mrs. Zerida Ntontori.

## ACKNOWLEDGMENT

All praises and gratitude to the Almighty GOD.

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| HIV: | Human Immune Virus |
| AIDS: | Acquired Immune Deficiency Syndrome |
| TB: | Tuberculosis |
| WHO: | World Health Organization |
| EMB: | Ethambutol |
| INH: | Isoniazid |
| RMP: | Rifampicin |
| PZA: | Pyrazinamide |
| TEM: | Traditional Eye Medicines |
| CNS: | Central Nervous System |
| mg/kg: | milligrams per kilogram |
| SSA: | Special Somatic Afferent |
| TON: | Toxic Optic Neuropathy |
| MDR: | Multidrug Resistant |
| PBS: | Phosphate Buffer Saline |
| µm: | Micrometer |
| mm: | Millimeter |
| CSF: | Cerebrospinal Fluid |
| H & E: | Hematoxlyin and Eosin |
| LFB: | Luxol Fast Blue |
| KIU: | Kampala International University |
| ANOVA: | Analysis Of Variance |
| ATP: | Adenosine Triphosphate |
| TE: | *Trifolium* Extract |
| AMP: | Adenosine Monophosphate |
| ROS: | Reactive Oxygen Species |

## ABSTRACT

**Introduction:** Ethambutol (EMB) has been discovered as an anti-tuberculosis drug since 1960, and is now very important in treatment of multidrug resistant tuberculosis which is on a rise due to emergence of Human Immunodeficiency Virus. However, EMB has been associated with severe side effects including optic neuropathy with no preventive and treatment measures.

**Purpose:** To establish the protective potential of *Lantana trifolium* ethanolic extract against EMB induced optic nerve changes.

**Materials and Methods:** Experimental design involving 25 male adult Wistar rats of 110-130g average weight, divided into five groups each comprising five animals. All rats were fed on standard commercial rat pellets and water *ad libitum* for five weeks. Group A, the negative control received distilled water. Group B, the positive control was treated with EMB 100 mg/kg/day. Test groups C, D, and E were treated with 25, 50, and 100 mg/kg/day of *trifolium* Extract (TE) respectively, one hour before administering 100 mg/kg/day of EMB. Visual acuity during the 1$^{st}$, 3$^{rd}$, and 5$^{th}$ week was determined by the mean escape latencies obtained using a modified Moris water maze. Optic nerves were macroscopically examined for gross morphological changes. The optic nerves were excised, processed and stained using Luxol fast blue, Hematoxlyin and Eosin for histological studies. A light microscope (×40) was used to examine optic nerve histological changes and ImageJ for analysis.

**Results and Discussion:** *Lantana trifolium* ethanolic extract had a dose dependent protective potential against EMB induced changes. This was shown by the significant difference in visual acuity during the 5$^{th}$ week. There was a significant increase in the escape latencies of positive control group (9.65±1.22) when compared with those of the negative control group (4.35±0.50), EMB +50mg/kg TE group (4.85±0.65), and EMB + 100mg/kg TE group (3.6±0.38). The neuroprotective effect was further shown by the significant difference in histological changes. The protective potential of *Latana trifolium* is likely due to the anti-oxidative and anti-inflammatory activities.

**Conclusion and Recommendation:** *Lantana trifolium* ethanolic extract has a dose dependent neuroprotective potential against EMB induced changes in the optic nerve. Since the exact phytochemical component and mechanism of action responsible for this effect are not known, further studies to find this out are strongly recommended.



**Key Words:** Ethambutol, Optic Nerve, *Lantana trifolium*, Optic Neuropathy.

# CHAPTER ONE
# INTRODUCTION

## 1.1 Background to the study

Ethambutol (EMB) is one of the first line drugs for treating tuberculosis (TB) (Biadglegne *et al.,* 2014), which affects one-third of the population worldwide (WHO, 2015). Despite the discovery of very effective drugs against it, TB is still a serious health challenge in developing countries including Uganda (Tola *et al.,* 2015). The treatment regimen for TB comprise of multiple drugs including Ethambutol (EMB), Isoniazid (INH), Rifampicin (RMP) and Pyrazinamide (PZA) for a minimum period of 6 months (Kuaban *et al.,* 2015). The development of multidrug resistant (MDR) TB along with Human Immunodeficiency Virus (HIV)/Acquired Immunodeficiency Syndrome (AIDs) condition have greatly increased the incidence of TB, making it a primary public health threat (McDonald *et al.,* 2015). EMB broadens the resistance spectrum and prevents development of resistance to companion drugs (Lacoma *et al.,* 2015). Regardless of these desirable characteristics, EMB is known to cause changes in the optic nerve that result into optic neuropathy (Wang *et al.,* 2012). This was established soon after its discovery in 1960s as a promising agent to treat tuberculosis (Forbes, Kuck and Peets, 1962).

Optic neuropathy, a major side effect of EMB is as a result of damage to the optic nerve (Yu-Wai-Man and Griffiths, 2013). This is one of the most serious conditions because of inability of the optic nerve fibres to regenerate, posing a risk of permanent and potentially severe impairment of vision (Pernet and Schwab, 2014). The symptoms of EMB induced toxic optic neuropathy are painless, symmetrical and progressive (Rasool *et al.,* 2015). Including blurring of vision, decrease in visual acuity, disturbances in color perception, bitemporal and centrocaecal visual field scotoma defects (Han *et al.,* 2015). The condition is underdiagnosed and the diagnosis is mostly done when recovery of vision is impossible (Jeanjean and Dupeyron, 2014). Other than stopping the drug use, no specific treatment is available for the EMB induced optic nerve changes (Rasool *et al.,* 2015).

EMB toxicity is classically described as reversible on discontinuation with complete recovery over period of weeks to months (Holla *at al.,* 2015). However worsening of vision after EMB

discontinuation is also documented (Garg *et al.,* 2015). There are reports of some patients who develop severe, irreversible vision loss despite frequent monitoring and standard dosages (Chen *et al.,* 2015). Worldwide a 100,000 people are estimated to lose vision completely each year due to the use of EMB (Bourne *et al.,* 2013). This number may increase due to prolonged use of antiTB drugs in HIV/AIDs patients and those with relapses. It is therefore necessary that studies to find a remedy for the toxic effects of EMB are carried out, of which *Lantana trifolium* leaf extract can be one of the remedies.

In Uganda, leaves of *Lantana trifolium* are traditionally used to treat eye conditions such as blindness, glaucoma, conjunctivitis, and bacterial and viral eye infection (Ocheng *et al.,* 2014). *Lantana trifolium* known as Kayukiyuki (Luganda), Omuhuuki (Runyankole) belongs to the family of Verbenaceae (Nalubega *et al.,* 2011). It is a three leafed, scrambling, evergreen shrub growing up to 3 meters tall in all subtropical and tropical regions (Schemske, 1976). The few studies that have been carried out on the chemistry of *Lantana trifolium* show that its biological activities are associated with its components mainly the phenols, phenylpropanoids, flavonoids and terpenoids (Imbenzi *et al.,* 2014).

**1.2 Problem Statement**

Ethambutol induces changes in the optic nerve that result into visual impairment. This is a progressive painless condition that is mostly diagnosed when recovery is impossible (Jeanjean and Dupeyron, 2014). Although EMB induced changes are often reversible when EMB is discontinued, in some patients recovery is incomplete (Osaguona *et al.,* 2014 and Pradhan *et al.,* 2010). However permanent visual impairment has also been reported in some patients (Osaguona *et al.,* 2014). In Uganda TB burden is still high (Kirenga *et al.,* 2015), the use of EMB is frequent but the eye tests before and during its use as recommended by WHO are rarely done due to limited resources (Mustak *et al.,* 2013).

Regardless of realizing that EMB causes changes in the optic nerve soon after its discovery in 1960s. It is still used up to date because EMB has no alternative substitute (Chen *et al.,* 2015). More so there are no preventive and treatment measures for EMB induced optic nerve changes (Yang *et al.,* 2016). Therefore means of protecting the optic nerve against EMB toxicity need to

be sought. This study established if *Lantana trifolium* ethanolic extract could protect the optic nerve against ethambutol induced changes.

### 1.3 Purpose of the Study

To establish the neuroprotective potential of *Lantana trifolium* ethanolic extract against ethambutol induced changes in the optic nerve.

### 1.4 Specific Objectives

1. To evaluate the effect of *Lantana trifolium* ethanolic extract on ethambutol induced changes in visual acuity.
2. To determine the effect of *Lantana trifolium* ethanolic extract on ethambutol induced changes on the gross morphology of the optic nerve.
3. To investigate the effect of *Lantana trifolium* ethanolic extract on ethambutol induced changes in the histology of the optic nerve.

### 1.5 Research Hypothesis

1. Ho: *Lantana trifolium* ethanolic extract does not have an effect on ethambutol induced changes in visual acuity.

   $H_1$: *Lantana trifolium* ethanolic extract has an effect on ethambutol induced changes in visual acuity.
2. Ho: *Lantana trifolium* ethanolic extract does not have an effect on ethambutol induced changes on the gross morphology of the optic nerve.

   $H_1$: *Lantana trifolium* ethanolic extract has an effect on ethambutol induced changes on the gross morphology of the optic nerve.
3. Ho: *Lantana trifolium* ethanolic extract does not have an effect on ethambutol induced changes in the histology of the optic nerve.

   $H_1$: *Lantana trifolium* ethanolic extract has an effect on ethambutol induced changes in the histology of the optic nerve.

### 1.6 Scope of Study

This study included determining the protective effects of *Lantana trifolium* extract on visual acuity, gross morphology, and histology of the optic nerve in ethambutol induced optic neuropathy of Wistar rats.

### 1.7 Justification

Tuberculosis might not be eradicated in the near future, thus EMB will continue to be used until there is a better alternative drug. In developing countries Uganda inclusive EMB is frequently used but routine eye tests are rarely done. It is therefore necessary that preventive measures are sought. Since *Lantana trifoliim* is widely used traditionally in Uganda to treat eye conditions (Ocheng *et al.,* 2014), it is important that studies are carried out to determine its effects on EMB induced changes in the optic nerve. This can then be a basis for other researches to determine its mechanism of action and with time may be help the community improve the knowledge of its use. The high doses of the plant extract administered to the animals can reflect the effect of the extract in humans.

### 1.8 Conceptual Framework

Optic nerve is vulnerable to ethambutol toxicity. This causes changes in the optic nerve that result into optic neuropathy. This affects visual acuity, gross morphology and histology of the optic nerve. The study determined the neuroprotective potential of *Lantana trifolium* ethanolic extract against ethambutol induced changes in the optic nerve.

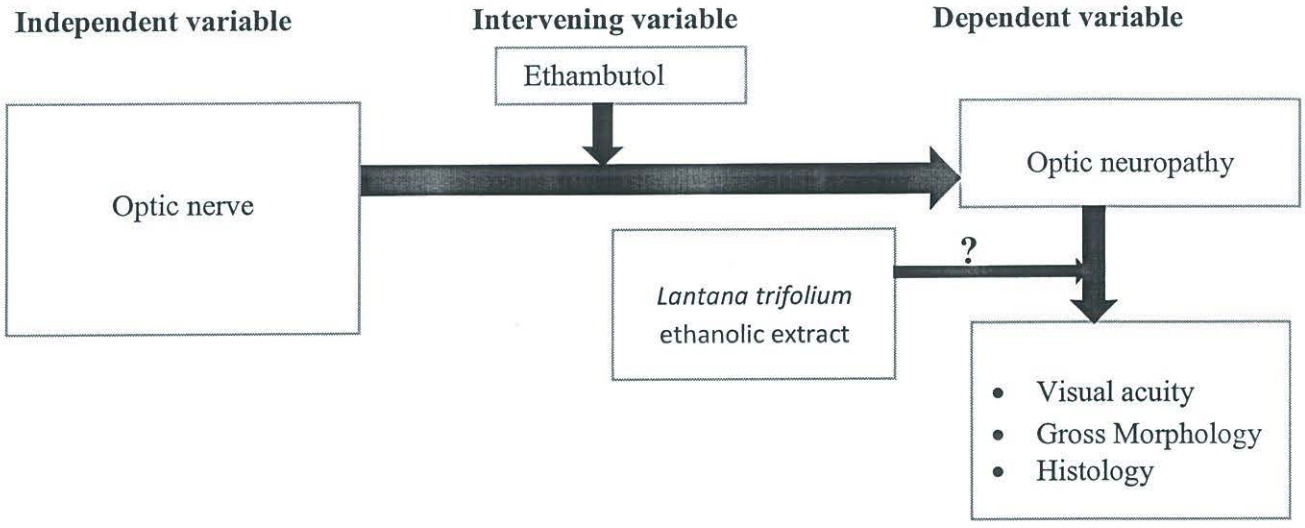



**Figure 1: A Diagrammatic Representation of the Conceptual Framework.**

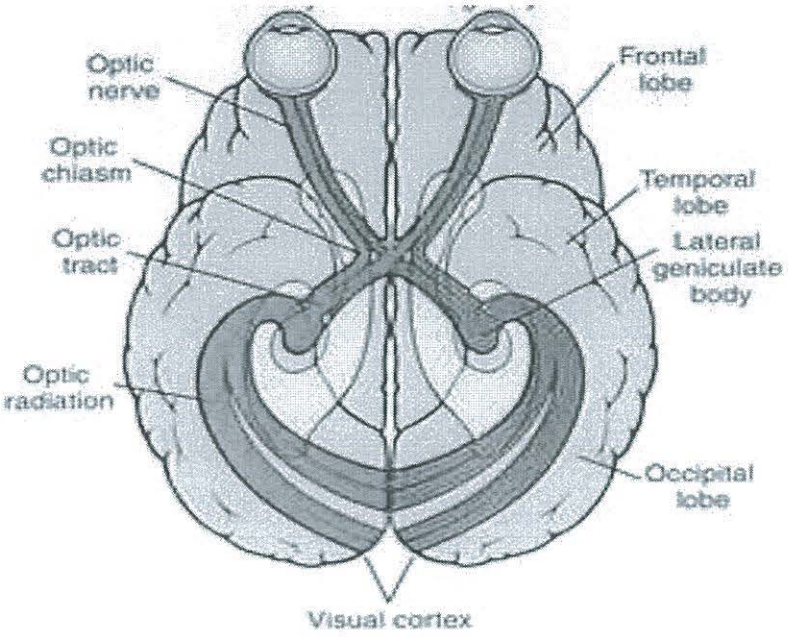
# CHAPTER TWO
# LITERATURE REVIEW

## 2.1 Visual acuity

Visual acuity is the sharpness of vision. Vision is a special sense which perceives the form, colour, size, movement, and distance of objects (Gori *et al.,* 2012). It consists of several visual functions, including; visual acuity, visual field, contrast sensitivity, colour perception, binocular vision, stereoscopic vision and visual adaptation to different luminance levels (Rezaul and Kojima, 2010). The visual information is relayed from the eye to the visual cortex by different structures that form the visual pathway (Prasad and Galetta, 2011). These include; eye, optic nerve, optic chiasma, optic tract, lateral geniculate body, optic radiation, visual cortex and the visual association cortex (Hofer *et al.,* 2010).

The process of vision starts with the eye, where the light is focused onto the retina and is transduced into electrical impulses that are conducted by the optic nerve to the optic chiasma (Melloni, 1971). At the chiasma some of the nerve fibres carrying visual information from each eye cross over (Cowey and Franzini, 1979). The information then travels through the optic tracts into the lateral geniculate nucleus where it is grouped according to the eye the information originates from and the stream (Dan *et al.,* 1998), whether it is from the parvocellular or magnocellular stream (Andrews *et al.,* 1997). The information is then taken by the optic radiations to the visual cortex where it is interpreted as a visual image (Kastner and Ungerleider, 2000).

The visual pathway organizes the flow of information so that the correct signal reaches the visual cortex (Spiridon and Kanwisher, 2002). Damage to any part of the visual pathway results into visual impairment (Biousse, 2007). The severity of the impairment depends on which part is affected (Rowe *et al.,* 2009). A damage anterior to the optic chiasma cause vision impairment in the eye on that same side, at the chiasma the impairment of vision is in both visual fields of both eyes (Monteiro *et al.,* 2010). Damage posterior to the optic chiasma results in nasal visual field defect of the opposite eye and temporal visual field defect of the same side (Prasad and Galetta, 2011).




Source: http://img.tfd.com/MosbyMD/thumb/visual-pathway.jpg

**Figure 2: A Diagrammatic Representation of the Visual Pathway.**

### 2.2 Optic Nerve

The optic nerve is a cranial nerve II, it transmits visual information in electrical signal form from the retina to the brain (Kuffler, 1953). It transmits all the visual information including visual acuity, contrast and color perception from the retina to the visual cortex (Libedinsky and Livingstone, 2011) where the visual image is formed (Prasad and Galetta, 2011). It also conducts the visual impulses that are responsible for light and accommodation reflex (De Lima *et al.,* 2012). The optic nerve is considered part of the central nervous system because it is derived from an outpouching of the diencephalon during embryonic development (McKay, 1997).

The pair of the optic nerves joins to form the optic chiasma (O'Connell, 1973), where decussation of all the ganglion cell axons arising from the nasal half of the retina to the opposite optic tract takes place (Reese *et al.,* 1991). All ganglion cell axons arising from the temporal half of the retina proceed without decussating and join the optic tract of the same side (Baker *et al.,* 1993). Most of the axons of the optic nerve terminate in the lateral geniculate nucleus of the thalamus from where information is relayed to the visual cortex for vision by way of the optic radiation fibers (Sherbondy *et al.,* 2008). Other axons terminate in; the pretectal nuclei of the midbrain and are involved in reflexive eye movements (Soto *et al.,* 2008); the suprachiasmatic

nucleus of the hypothalamus and are involved in regulating the sleep wake cycle (Folgueira *et al.,* 2008); the superior colliculus of the midbrain and are involved in vision associated with somatic reflexes (Redgrave *et al.,* 2010).

The optic nerve injuries are the most overwhelming conditions in the visual pathway (Maresca *et al.,* 2013). They result into abnormal pupillary reflex, retinal ganglion cell degeneration, visual field impairment and complete blindness (Nusbaum *et al.,* 2015). These injuries can be due to glaucoma, trauma, inflammation, ischemia, compression from tumors, congenital complications or toxicity like ethambutol induced optic neuropathy (Guy *et al.,* 2014).

### 2.2.1 Gross Morphology of the Optic Nerve

The optic nerve is a cylindrical white matter tract of the Central Nervous System (CNS) (Lambert *et al.,* 1987). It extends from the eye globe to the optic chiasm (Kruger *et al.,* 1998). Its average length is 50 mm, but greatly varies even in a pair of an individual (Wheeler-Kingshott *et al.,* 2002). Anatomically the optic nerve is divided into four parts; the optic head, orbital part, intracanicular part, and cranial part (Pletcher *et al.,* 2006). The diameter of the human optic nerve tract increases from 1.18–1.75mm within the eye globe, to 3-4mm in the orbit, to 4-7mm within the cranial cavity (Watanabe *et al.,* 2008).

The optic nerve is protected by the meningeal sheath, pia, arachnoid, and dura maters throughout its course (Sturrock, 1987). The subarachnoid space contains cerebrospinal fluid (CSF) that cushions the nerve from mechanical injury (Hansen and Helmke, 1997), but if CSF pressure increases, it causes intracranial hypertension which can lead to papilledema (Padhye *et al.,* 2013). A condition in which there is inflammation and damage of the optic nerve (Friedman, 2014). Papilledema results into visual impairment, including smaller visual field, blind spots, double vision, and short temporary episodes of blindness (Bidot *et al.,* 2015).

The optic nerve leaves the bony orbit through the optic canal within the sphenoid bone, and enters the cranial cavity (Chou *et al.,* 1995). It runs along the surface of the middle cranial fossa, adjacent to the pituitary gland (Shah, 1999). Optic nerve function is compromised in fractures across the skull base and optic canal stenosis (Hathiram *et al.,* 2011). The latter is common in fibrous dysplasia associated with the McCune–Albright syndrome that usually involves the

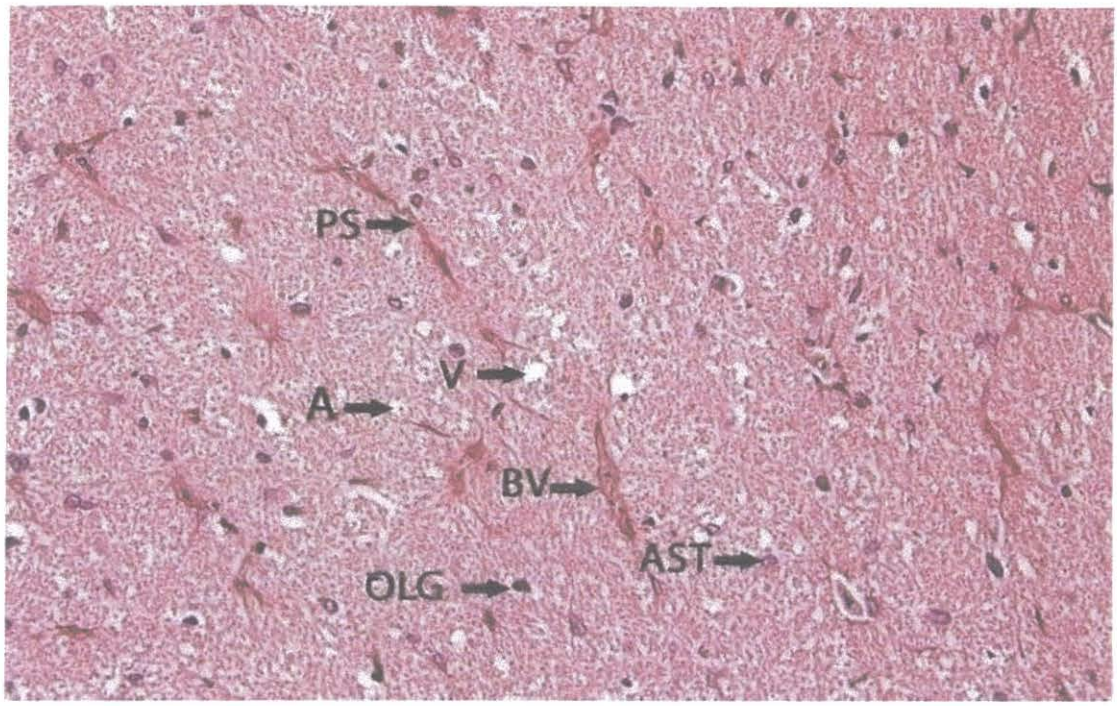
sphenoid bone (De Araújo *et al.,* 2012). Visual function may be impaired when the optic nerve is impinged by an enlarged pituitary tumor (Kuwabara and Yuki, 2013). This is initially due to reversible physiological axonal conduction block but later demyelination, axonal degeneration and ischemia result into irreversible vision impairment (Scangas and Laws, 2014).

### 2.2.2 Histology of Optic Nerve

Optic nerve consists of the myelinated axons of the retinal ganglion cells (Ueda *et al.,* 1999). These axons are the special somatic afferent (SSA) fiber that conduct electrical impulses for the special sense of vision (Veraart *et al.,* 1998). Each human optic nerve consist 770,000-1.7 million retinal ganglion cell axons. Glial cells, oligodendrocytes, astrocytes, and microglia support the optic nerve fibers (Brooks *et al.,* 1999). Oligodendrocytes are the major neuroglia in the optic nerve. These cells are responsible for production of myelin sheaths of the nerve fibers which facilitates the fast conduction of axons (Dougherty *et al.,* 2000). They lie in regular interfascicular rows along the long axis of the nerve (Kalsi *et al.,* 2004). They have a spherical shaped cell body with a central small nucleus. Their cell processes are fewer and more delicate than those of astrocytes (Zhu *et al.,* 2007).

Astrocytes are interspersed between the series of oligodendrocytes. They function in remodeling of neural tissues during development and in disease (Hernandez, 2000). These cells have a star shape appearance with an irregularly shaped nucleus (Hishikawa *et al.,* 2005). Microglia, the macrophages of the optic nerve are distributed throughout the nerve tissue adjacent to the blood vessels and the nerve bundles (Chen *et al.,* 2002). In normal optic nerves microglia cells have a stellar shape with a small nucleus and cell body having numerous ramified processes (Ayoub and Salm, 2003). These cells are rapidly activated into antigen presenting cells in response to optic nerve damage and may undergo mitosis to become phagocytic cells (Mack *et al.,* 2003).

The optic nerve is affected by all the pathological conditions that affect the CNS including multiple sclerosis, demyelination, toxicity and inflammation (Jger and Miszkiel, 2008). More than 80% of patients with multiple sclerosis develop visual impairment characterized by decrease in visual acuity and contrast sensitivity (Bermel and Balcer, 2013). Since nerve fibers are unable to regenerate (Münzel *et al.,* 2014), damage to the optic nerve results irreversible visual impairment.



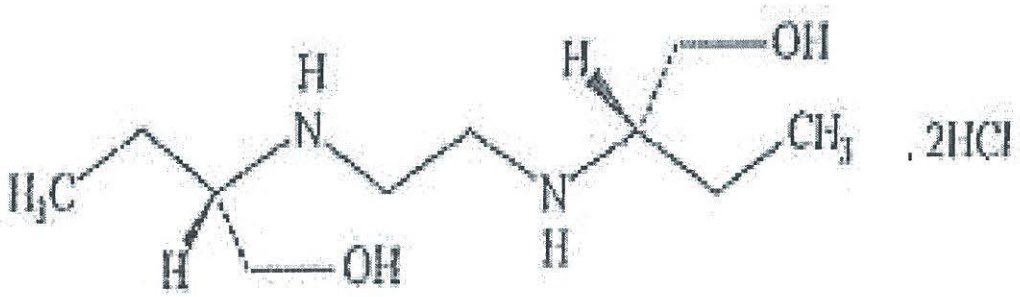

Source: Zubair *et al.*, (2009)

**Figure 3: Photomicrograph of Optic Nerve.**

Showing astrocytes (AST) pial septa (PS), blood vessels (BV), optic nerve fibres (A), vacuoles (V) and oligodendrocyte (OLG) H & E stain, X 200.

### 2.3 Ethambutol Induced Optic Neuropathy

Ethambutol (EMB) is an antibiotic belonging to the amino-alcohol group (Deng *et al.*, 1995). It is an efficacious first line antituberculosis agent (Gwaza *et al.*, 2014). EMB broadens the resistance spectrum and prevents development of resistance to companion drugs (Mao *et al.*, 2015). Its chemical formula is 2, 2' Ethylenediimino-di-1-butanol dihydrochloride (Freeland *et al.*, 1979). This drug is available on the market in a tablet form taken orally with or without food (Kinoshita *et al.*, 2012). EMB is readily absorbed from the gastrointestinal tract (Pan *et al.*, 2013) and penetrates into the lung, liver, cerebrospinal fluid and brain rapidly (Donald, 2010). It reaches the peak plasma concentration of 4 to 5 mg/L within 2 to 4 hours (Jönsson *et al.*, 2011) and has a half-life of 3–4 hours (Xu *et al.*, 2013). Both unchanged and inactive hepatic metabolites of EMB are excreted from the body through the kidneys (Hall *et al.*, 2012).

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http://www.newdruginfo.com/pharmacopeia/usp28/v28230/uspnf/pub/images/v28230/g-331.gif

**Figure 4: Chemical Structure of Ethambutol.**

Although EMB is known to be bacteriostatic against Mycobacterium tuberculosis, its exact mechanism of action has not been confirmed (Palomino and Martin, 2014). However the chelating effects of EMB and its metabolites that interfere with several metal containing enzymes resulting into inhibition of mycobacterial cell wall synthesis has been suggested (Neuropathy *et al.,* 2016). It has been stipulated that its toxicity involves a similar mechanism (Rasool *et al.,* 2015). EMB is known to cause changes in the optic nerve that results into toxic optic neuropathy (TON) (Talbert and Sudan, 2010). This was established soon after its discovery in 1960s. Forbes, Kuck and Peets (1962) reported EMB induced optic neuropathy in 50% of patients receiving 60–100 mg/kg/d of EMB. This toxicity is generally described as dose and duration dependent (Talbert and Sudan, 2010), but there is no effective dose that is entirely free from the threat of toxicity (Zelefsky *et al.,* 2012). The toxicity is observed at a dose as low as 12.3 mg/kg (Guerrero *et al.,* 2013). According to World Health Organization (WHO) the initial treatment dose should be 15mg/kg daily or 25 mg/kg three times weekly for the first 2 months of treatment and a retreatment dose of 25 mg/kg daily. Although the onset of EMB induced toxic optic neuropathy symptoms are usually delayed (Zhao *et al.,* 2013), occurring at least 1.5 months after therapy initiation (Kervinen *et al.,* 2013). A number of cases of toxicity occurring as early as after less than six days of treatment have been reported (Yang *et al.,* 2016).

Optic neuropathy, the most important toxic effect of EMB (Grzybowski *et al.,* 2015) manifests as bulbar or retrobulbar neuritis, the latter being the most common type (Mustak *et al.,* 2013).

This is a painless symmetrical progressive condition (Han *et al.,* 2015). The earliest sign of EMB induced optic neuropathy is loss of color perception, particularly red and green (Garg *et al.,* 2015). There is a decrease in visual acuity and contrast sensitivity (Han *et al.,* 2015). Central scotomas are the common visual field defect (Kandel *et al.,* 2012), but bitemporal defects and peripheral field constriction have also been reported (Kho *et al.,* 2011). Pupillary abnormalities and visual evoked potential are used to confirm the diagnosis of the EMB induced optic neuropathy (Jayaraman *et al.,* 2014). The subclinical toxicity can be detected by measuring visual acuity (Grzybowski *et al.,* 2015). There is no specific treatment for this condition other than stopping the use of the drug (Rasool *et al.,* 2015). Although EMB induced optic neuropathy is generally said to be reversible when EMB is discontinued (Osaguona *et al.,* 2014), permanent vision impairment has been reported in some patients. In some cases many patients do not recover completely (Yang *et al.,* 2016).

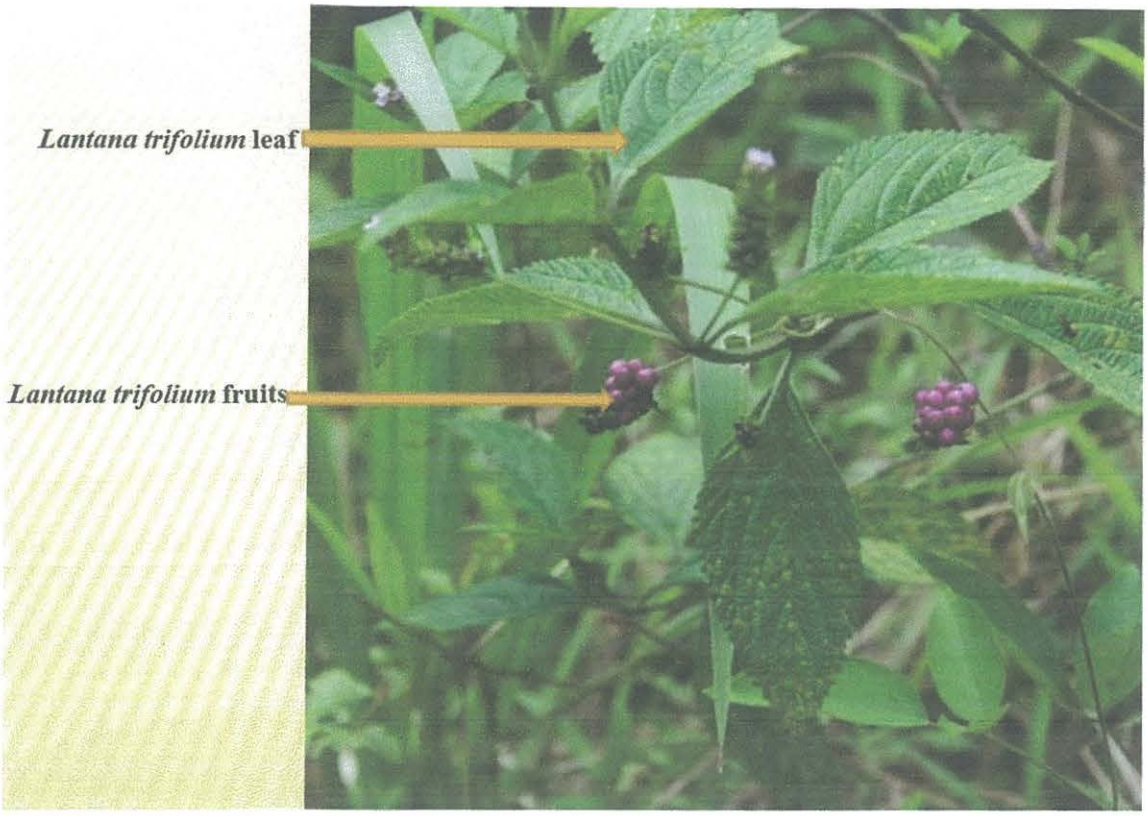
Optic nerve fibers, along with the glial cells are vulnerable to EMB toxicity (Crawford *et al.,* 2014). Ethambutol toxicity causes inflammation and vacuolation of the optic nerve axons (Guillet *et al.,* 2010a). This results into lesions and atrophy of the optic nerve axons (Payne *et al.,* 2012). Although there is a reduction noted in the optic nerve diameter, it has been found to be statistically insignificant in many studies (Masvidal *et al.,* 2010). Demyelination of optic nerve axons during EMB toxicity has been reported in some studies (Osaguona *et al.,* 2014). Oligodendropenia was reported following use of toxic doses of EMB in experimental animals (Mustak *et al.,* 2013). Different experimental animals when treated with toxic doses of EMB revealed multiple vacuoles, axonal fragmentation, inflammatory changes, demyelination and central necrosis in the optic nerve (Osaguona *et al.,* 2014).

## 2.4 *Lantana trifolium*

Traditional eye medicines (TEM) are commonly used to treat different eye diseases worldwide (Julio *et al.,* 2010). *Lantana trifolium* is widely used to treat many eye conditions such as blindness, glaucoma, conjunctivitis, and bacterial and viral eye infection (Nalubega *et al.,* 2011). It is also used to treat epilepsy, infant cerebral malaria, asthma, sinusitis, menstrual pains, mental illiness, sickle cell anaemia, stomachache and skin conditions (De Sena *et al.,* 2012).

Phytochemical composition of *Lantana trifolium* has not been studied extensively. However previous studies have reported that the plant contains phenols, phenylpropanoids, flavonoids and terpenoids (Imbenzi *et al.,* 2014). The few studies that have been carried out previously suggest that *Lantana trifolium* is pharmacologically active at doses as low as 10mg/kg and is reasonably safe (Julio *et al.,* 2010). No toxic effects have been observed at doses as high as 3200mg/kg (Imbenzi *et al.,* 2014). *Lantana trifolium* is known for its anti-inflammatory properties (Silva *et al.,* 2005). Previous ethno-medicinal studies about this plant have reported that its extract inhibited carrageenan and histamine induced rat paw edema (Uzctegui *et al.,* 2004). The anti-inflammatory effect of extract is reported to be possibly due to histamine reduction (Julio *et al.,* 2010). The extract also has a small but significant increase in the latent response period for rats subjected to the hot plate, this shows a weak analgesic effect of the extract (Salabarría *et al.,* 2009). Since EMB induced optic neuropathy causes inflammation of the optic nerve fibers, *Lantana trifolium* ethanolic extract may be helpful in preventing it.

In a study using an open field method *Lantana trifolium* produced an intense sedative effect in all animals after one hour, that was reported as reduction of walked squares (Julio *et al.,* 2010). This effect is attributed to the flavonoids and phenylpropanoids in the extract (Santana *et al.,* 2010). Moreover flavonoids are known to be effective at different receptor systems in the CNS, of which optic nerve is a part (Kumar and Pandey 2013). *Lantana trifolium* extract has anti-oxidative and free radical scavenging activities which are due to the high amounts of phenols it contains (Salabarría *et al.,* 2009). The extract is able to convert free radicals to more stable compounds by proton donation, thus terminating the chain reactions (Perumal *et al.,* 2012). Using the same mechanism *Lantana trifolium* could inhibit vacuole formation and cell death during EMB induced optic neuropathy.




Source:

http://tropical.theferns.info/plantimages/3/8/385fb91a4914409c86270872a32ae0f1b1b20379.jpg

**Figure 5: *Lantana trifolium* Plant.**

# CHAPTER THREE
# RESEARCH METHODOLOGY

## 3.1 Study Design

This was an experimental study in which the effects of *Lantana trifolium* ethanolic extract on ethambutol (EMB) induced changes were investigated, using quantitative and qualitative methods.

## 3.2 Study Population

The study was conducted on male adult Wistar rats of 110-130g average weight obtained from the animal facility at Kampala International University (KIU).

## 3.3 Sample Size

The minimum number recommended for animal experiments is three (3) in each group. In this study to increase the chance of obtaining significant results five (5) animals were used in each of the five groups. Therefore a total of twenty five (25) male adult Wistar rats were used in the study. A "resource equation" method was used to determine if this sample size was adequate.

"Resource equation" method (Walter *et al.*, 1998);

E = Total number of animals – Total number of groups

(E is the degree of freedom of analysis of variance)

Total number of animals = 25

Total number of groups =5

E = 25 – 5

E = 20 (Sample size which retains E between 10 and 20 is considered adequate).

Thus a sample size of twenty five (25) animals was used in this study.

## 3.4 Preparation of the Extract

### 3.4.1 Plant

Leaves of the plant were collected and packed in a black polythene bag, from Bushenyi district in western Uganda. A sample of the fresh and a shade dried plant was taken to Makerere University Herbarium for identification by a qualified Botanical Taxonomist. It was identified as *Lantana trifolium* and given a collector number OE #001.

### 3.4.2. Preparation and Storage of the Extract

Leaves of *Lantana trifolium* were properly washed with tap water before shade drying, and then ground using a motor and pestle. The dry powdered material was put in the thimble and loaded into a Soxhlet apparatus. Ethanol was added to a round bottom flask that was attached to the extractor and the flask was heated to evaporate, moving through the apparatus to the condenser. The condensate dripped into the reservoir containing the thimble. The temperature was maintained at 80 °C until no liquid flowed into the flask (Luque and Priego-capote, 2010).

The extract from the round bottom flask was filtered through a Watmann filter paper (125 mm) and concentrated using a rotary evaporator maintained at 40 °C. Using a water bath, the concentrated extract was evaporated to dryness, weighed and stored in refrigerator at 4°C.

## 3.5 Animal Studies

### 3.5.1 Experimental Animals

Male adult Wistar rats used in this experiment were housed in cages, exposed to 12 hour of dark and light cycles. They were fed on standard commercial rat pellets (crude protein: 23 %, crude fat: 5%, crude fiber: 7%, ash: 8%, calcium: 2 % phosphorus: 0.9%, sodium: 1%, moisture: 12%) (Reeves *et al.,* 1993), and provided with tap water *ad libitum*. The rats were weighed to determine the doses.

### 3.5.2 Animal Grouping

Animals were randomly divided into five groups, A, B, C, D, and E of five rats each. Group A and B served as control, whereas group C, D and E served as test. **Group A,** the negative control group received distilled water. **Group B**, the positive control group received EMB at a dose of 100mg\kg\day orally for five weeks. **Groups C, D and E** received *Lantana trifolium* ethanolic extract at doses of 25mg\kg\day, 50mg\kg\day and 100mg\kg\day orally respectively one hour before orally receiving EMB at doses of 100mg\kg\day for five weeks.

### 3.5.3 Administration of Ethambutol and *Lantana trifolium* Ethanolic Extract

Ethambutol (COSMOS LIMITED, ETHAM® TABLETS) at doses of 100mg/kg/day induces optic neuropathy in different experimental animals (Kinoshita *et al.,* 2012). Ethambutol and plant extract were each dissolved in distilled water and then administered once daily using oral gavage. EMB was administered one hour after *Lantana trifolium* administration. The dose of EMB and plant extract given to each of the animals was calculated using the standard formula below.

$$\text{Dose given} = \frac{\text{dose (mg/kg) x animal weight (grams)}}{\text{Concentration (mg/ml) x 1000.}}$$ (Ninsiima *et al.,* 2016)

### 3.6 Data Collection Technique

### 3.6.1 Visual Acuity Testing

A visible platform Morris water maze was used to determine the visual acuity. This device is based on the principle that rats can instinctively swim and can be trained to escape onto a visible platform. The water maze was a circular galvanized tank with white walls measuring 1.2 m in diameter and 0.6m in height, divided into four equal quadrants. Water at 25°c was used to fill the tank to a depth of 30cm. A circular unfixed platform of 10cm in diameter protruding 1cm above the water surface was used. A bright cloth was used to cover the protruding part of the platform to make it highly visible.

All the rats were submitted to pre-training and training session before visual acuity testing was done. During the pre-training session each rat would be placed into the water maze and then allowed to swim, locate and mount the visible platform placed in the center of one of the four quadrats. The rats were given one or more trials of 60 seconds time limit. After succeeding in finding and mounting the platform, the rats were trained for 2 days. In the period of training 4 trials were given to each rat on each day. On each trial the platform was moved to the center of a different quadrant, and the rats were released from different starting points and allowed to find and escape onto the platform. After mounting the platform, the rat would be left there for 15 seconds after which it would be removed. The rats would then be put into a holding cage containing pre-warmed towels for 1 minute till the start of the next trial. The rat that would fail to find the platform in 60 seconds would be guided to reach it, and this trial would be repeated. The time taken for the rat to find and mount the platform for each trial was recorded as the

escape latency (in seconds). Testing was done on day 3 during the 1st, 3rd, and 5th week of the experiment. Visual acuity was determined using the mean escape latencies (Prusky *et al.,* 2000).

### 3.6.2 Gross Morphological and Histological Analysis of the Optic Nerve

The animals were deeply anesthetized by placing them in a transparent air tight container having cotton soaked with 1.9% of ether. An incision was made in the skin overlying cervical region when the animals were unconscious. Using surgical blade, forceps and scissors for dissection the scalp was removed exposing the skull cap. This was detached from the dura mater and the calvarium lifted to expose the brain. The falx cerebri was pulled posteriorly, away from the crista galli. The frontal lobes were raised from the anterior cranial fossa, incised and removed to expose the optic nerves along with the chiasma (Heffner *et al.,* 1980).

#### 3.6.2.1 Gross Morphological Analysis of the Optic Nerve

Optic nerves were macroscopically examined and any changes observed recorded.

#### 3.6.2.2 Histological Analysis of the Optic Nerve

Both optic nerves were cleanly dissected, incised proximal to the optic chiasma and then removed along with the eye balls. They were fixed immediately with 4% paraformaldehyde in phosphate buffer saline (PBS). The leak proof containers with the tissues were transported to central diagnostic laboratory, Makerere University for processing. After 24 hours the nerves were removed and rinsed thoroughly using PBS in three washes and then post fixed in 2% osmium tetroxide in PBS for 2 hours. The optic nerves were excised from the eye balls and placed into tissue cassettes. These were placed into the tissue basket which was loaded into an automated tissue processor (Histokinette-SLEE MAINZ, MTP type). After which they were removed, washed with PBS and dehydrated in a series of 75%, 90%, and absolute alcohol. To be able to obtain transverse cross sections the optic nerves were placed vertically in the center of embedding moulds. These were then filled with molten paraffin wax and allowed to cool on a cold plate of embedding centre. The tissues blocks were the removed from the embedding moulds and the excess wax trimmed off using a blade.

#### 3.6.2.2.1 Hematoxlyin and Eosin (H&E) Staining Technique

This was used to demonstrate vacuoles in the optic nerve. Sections of 3µm thickness were cut from the cold tissue blocks using a rotatory microtome (SLEE MAINZ, CUT4062 model), and mounted onto salinized slides and stained using H&E stain (Kiernan *et al.*, 2010).

The slides were put in xylene for 3 minutes to remove paraffin wax and then put in two containers of absolute alcohol for 30 seconds each to remove the xylene. They were then transferred to 90% alcohol for 30 seconds and to 70% alcohol for 30 seconds and rinsed in distilled water. The clean slides were then put in Harris Hematoxylin for 9 minutes, washed thoroughly in tap water and then differentiated in acid alcohol solution for 15 seconds. The slides were washed using running tap water for 5 minutes and counter stained in Eosin solution for 1minute. Slides were washed in tap water until the excess eosin was removed. They were dehydrated through 95% and 100% alcohols, then cleared in xylene and a coverslip immediately put.

The slides were examined under a light microscope (Nikon Eclipse Ci, 104C type) using X40 objective and the changes recorded. Photomicrographs were taken using a mounted digital camera (Nikon digital sight DS, Fi 1) attached to a computer with software (NIS-Elements F3.00, SP7; Build 547). The photomicrographs were analyzed using ImageJ 2015 (Fiji). The numbers of vacuoles in the photomicrographs were counted after image acquisition, visualization, image processing, segmentation, and feature extraction.

#### 3.6.2.2.2 Luxol Fast Blue (LFB) Staining Technique

Luxol fast blue stain was used to stain the myelin sheath of optic nerve. Sections of 10µm thickness were cut from the cold tissue blocks using a rotatory microtome (SLEE MAINZ, CUT4062 model), and mounted onto salinized slides and stained (Lockard and Reers, 2009).

The slides were put in xylene for 3 minutes to remove paraffin wax and hydrated with 95% alcohol and then stained with LFB stain overnight at 60°C, rinsed in 95% alcohol and distilled water. The slides were then put in Lithium carbonate solution for 5 seconds, and then transferred to two containers of 70% alcohol for 10 seconds each. They were rinsed in distilled water. After

which the slide were rinsed in 70% alcohol for 10 seconds and then put in Eosin for 1 minute. They were then rinsed in distilled water and put in Cresyl violet for 1 minute. The slides were then rinsed in distilled water and dehydrated through 95% and 100% alcohols. The slides were cleared in xylene, after which a coverslip was put.

They were examined under a light microscope (Nikon Eclipse Ci, 104C type) using X40 objective and the integrity of the myelin sheath recorded. Photomicrographs were taken using a mounted digital camera (Nikon digital sight DS, Fi 1) attached to a computer with software (NIS-Elements F3.00, SP7; Build 547).

### 3.7 Data Analysis and Presentation

#### 3.7.1 Data Analysis

The escape latencies were entered into Microsoft excel and then exported to graph pad prisms V6 for analysis. The mean ± SEM escape latencies obtained were used to determine visual acuity. The optic nerves were macroscopically examined. The number of vacuoles observed in the photomicrographs were counted using ImageJ 2015 (Fiji) and entered into Microsoft excel. Using graph pad prisms V6, mean ± SEM of vacuoles were obtained. Analysis of variance (ANOVA) was applied to determine group mean differences between the control and test groups. A post-HOC Tuckey test was used to determine the significance of the differences in group means considering a p-value of $< 0.05$ to be statistically significant.

#### 3.7.2 Result Presentation

The results of quantitative data were presented in form of tables, graphs, photomicrographs and descriptions. The morphological data was presented in form of photographs and descriptions.

### 3.9 Limitations and Delimitations

Inaccessibility of a y-water maze to use for testing visual acuity. A modified Morris water maze was used in the study to determine visual acuity. Immunohistochemical techniques to provide more insight on EMB induced changes in the histology of the optic nerve were not carried out due to accessibility constraints. Therefore Luxol fast blue, hematoxlyin and eosin staining techniques were used to assess the histological changes.

### 3.8 Ethical Considerations

This study was aimed at finding means of protecting the optic nerve against EMB induced changes using *Lantana trifolium* ethanolic extract. EMB toxicity is not contagious, thus experimental animals were not harmful to people and environment. The study was approved by the Directorate of Postgraduate Studies and Research of Kampala International University Western Campus. All animal studies that were carried out in this experiment were conducted in accordance with "Guide for Care and use of Laboratory Animals" (National Research Council, 2010). A minimum number of animals were used in the study. These were housed in cages, exposed to 12 hour of dark and light cycles. The animals were fed on standard commercial rat pellets and provided with tap water *ad libitum.* Inflicting pain to the animals was minimized by sacrificing them under anaesthesia. At completion of the experiment, all the animal remains were incinerated. The ethanol used during extraction was recovered by collection into a conical flask, and was put into a container and stored for future use. The study resultss can be a basis to finding a remedy for ethambutol induced optic neuropathy. Results of this study may be help the community to improve the knowledge of *Lantana trifolium* uses.

## CHAPTER FOUR
## PRESENTATION AND INTERPRETATION OF RESULTS

*Lantana trifolium* showed a dose dependent neuroprotective effect against ethambutol (EMB) induced changes in the optic nerve of Wistar rats.

### 4.1 Effect of *Lantana trifolium* Ethanolic Extract on Ethambutol Induced Changes in Visual Acuity

Effect on visual acuity was determined by the escape latencies obtained using unfixed visible platform water maze. *Lantana trifolium* ethanolic extract showed a dose dependent improvement in the visual acuity. In the first week, there was no significant difference *(P > 0.05)* in the escape latencies of all the groups. Although the escape latencies of all the groups reduced during the 3rd week of the experiment, the differences when compared individually with those in the negative and positive control groups were found to be statistically insignificant *(P > 0.05)*. During the 5th week, the escape latencies of the positive control group and the group that received EMB and 25mg/kg of *trifolium* Extract (TE) increased (9.65±1.22) and (9.6±0.90) respectively. This was statistically significant *(P<0.05)* when compared against the negative control group (4.35±0.50). There was a further decrease in the escape latencies of remaining groups during the 5th week of the study. When compared against the positive control group, analysis of variance (ANOVA) showed a significant difference *(P<0.05)* in the escape latencies of the negative control group and the group treated with EMB and 50mg/kg of TE, but was more significant in the group treated with EMB and 100mg/kg of TE.

**Table 1: Mean Escape Latency**

| Experimental groups | N | Mean time (s) ± SEM Wk 1 | Wk 3 | Wk 5 |
|---|---|---|---|---|
| Negative control | 5 | 8.55±0.28 | 6±0.47 | 4.35±0.50## |
| Positive control | 5 | 8.8±2.02 | 7.9±1.34 | 9.65±1.22** |
| EMB + 25mg/kg TE | 5 | 8.65±1.70 | 7.8±1.15 | 9.6±0.90** |
| EMB +50mg/kg TE | 5 | 8.7±0.91 | 5.9±0.80 | 4.85±0.65## |
| EMB + 100mg/kg TE | 5 | 8.65±0.48 | 5.75±0.68 | 3.6±0.38### |

**KEY:** Wk = week; N = number of rats in each group; EMB = Ethambutol; TE = *trifolium* Extract. Multiple comparisons; against the negative control group **P<0.005;
Against positive control group, ##P < 0.005, ###P <0.0005.

### 4.2 Effect of *Lantana trifolium* Ethanolic Extract on Ethambutol Induced Changes on the Gross Morphology of the Optic Nerve

There was no significant difference observed in the gross morphology of the optic nerves from all the groups, all the nerves had a normal pinkish white color.

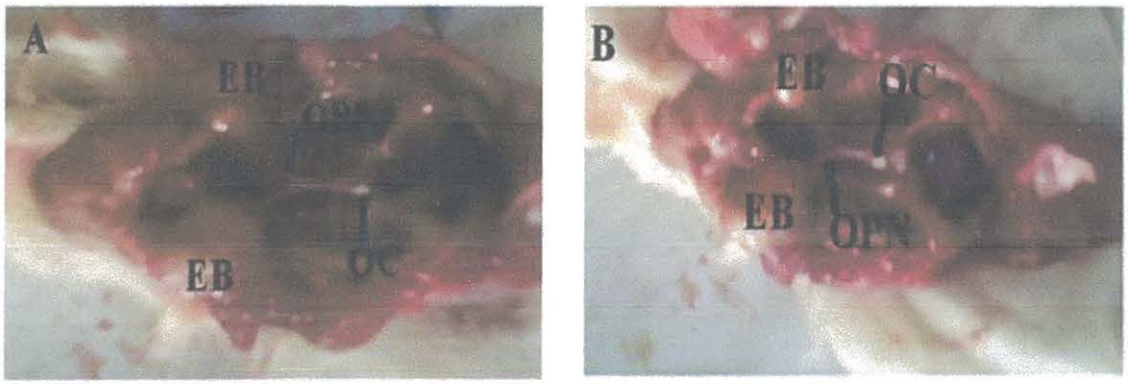



**Figure 6: Photograph Showing the Eye Ball (EB), and Arrows Pointing at Optic Nerve (OPN) and Optic Chiasma (OC) in Situ after Removing the Brain.**

KEY: Image A = negative control group and B = positive control group.

### 4.3 Effect of *Lantana trifolium* Ethanolic Extract on Ethambutol Induced Changes in the Histology of the Optic Nerve

During histological analysis cross sections of optic nerves were stained and changes in the histology determined by vacuolation and demyelination of the optic nerve.

### 4.3.1 Effect of *Lantana trifolium* Ethanolic Extract on Ethambutol Induced Optic Nerve Vacuolation

Varying numbers of vacuoles were observed in the optic nerves of all the groups. The number of vacuoles in positive control group (37.4±1.54) and; the group that received EMB and 25mg/kg of TE (36±1.30) were significantly high *(P<0.05)* when compared to the number of vacuoles in the negative control group (2.2±0.37). The number of vacuoles in the groups treated with EMB and 50mg/kg of TE (6.2±1.07) and; EMB and 100mg/kg of TE (5±0.71) were significantly few *(P<0.05)* when compared to those in the positive control group.

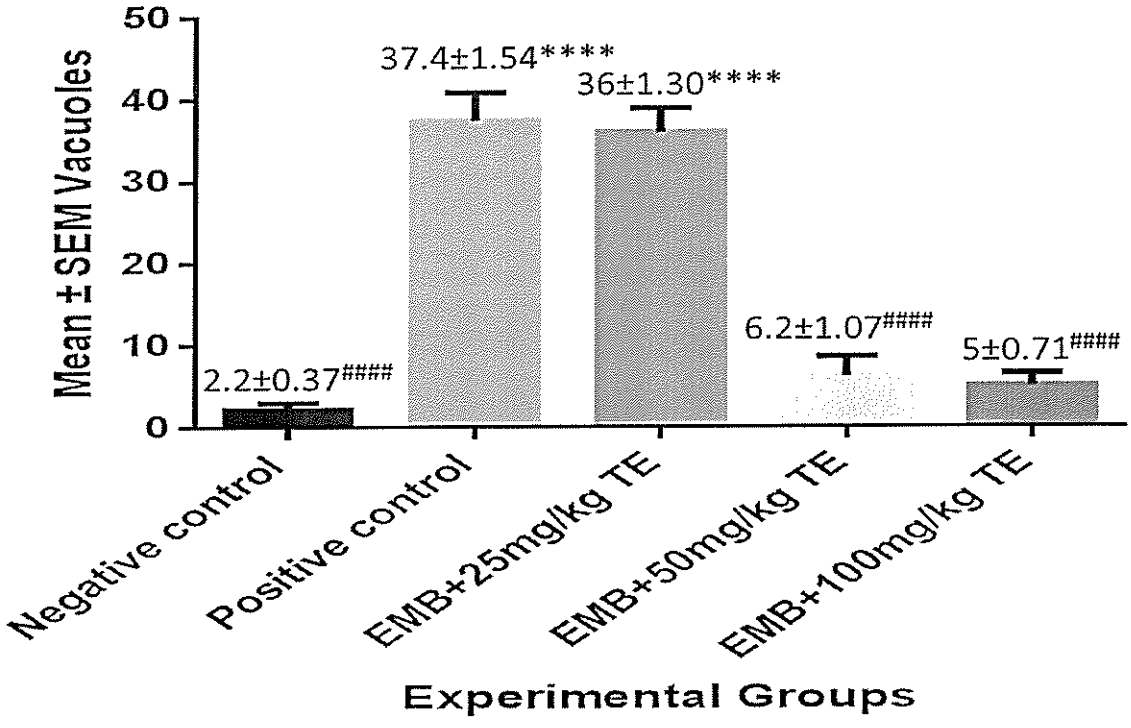



**Figure 7: A Bar Graph Showing Number of Vacuoles in the Optic Nerve Cross Section.**

KEY: EMB = Ethambutol; TE = *trifolium* Extract. Multiple comparisons; against the negative control group ****P<0.0001; against positive control group, ####P <0.0001.

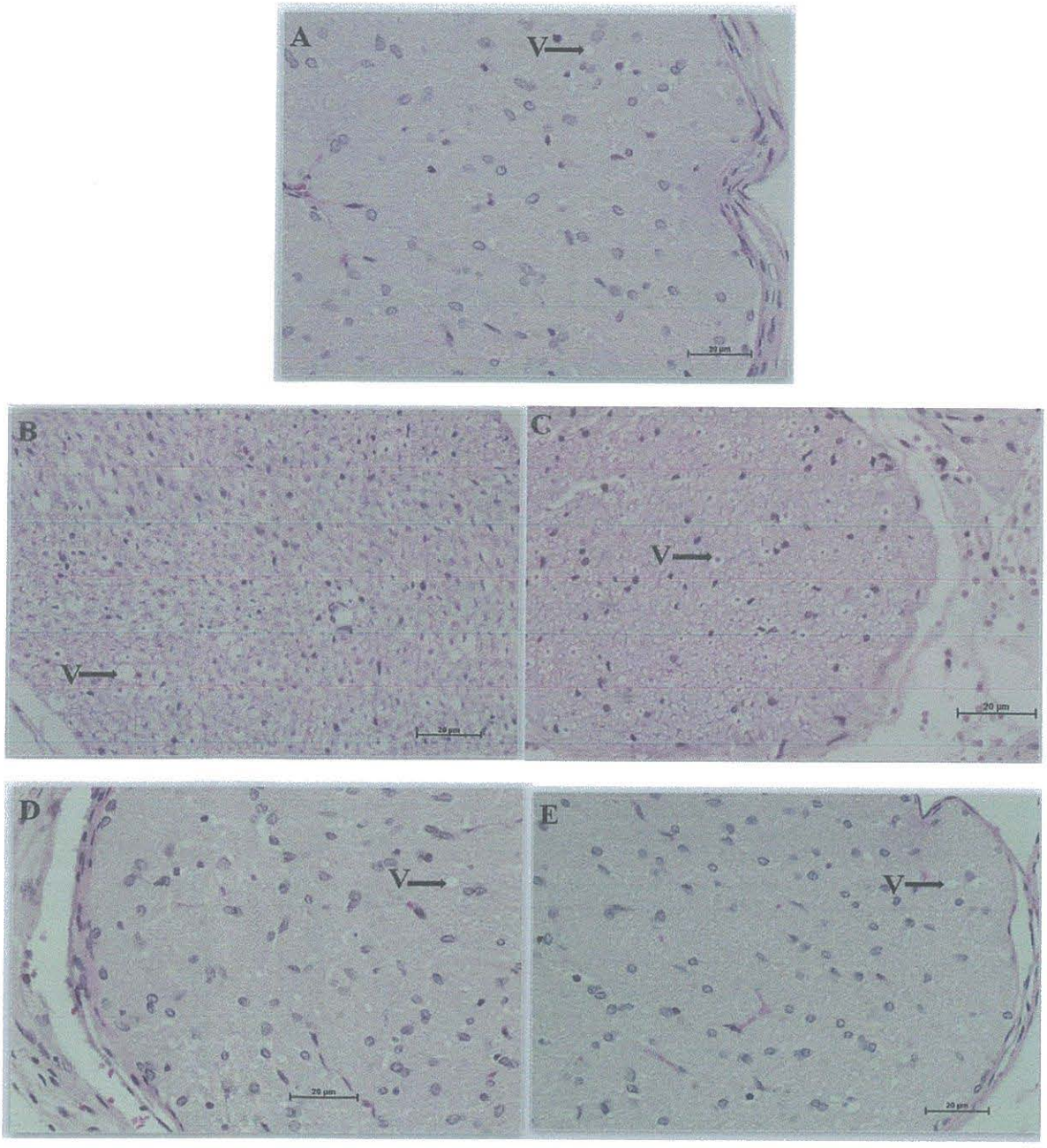



**Figure 8: Photomicrographs of Optic Nerve Cross Section Showing Vacuolation. H & E Stain, X40.**

KEY: Image, A = negative control group; B = positive control group; C = EMB + 25mg/kg TE group; D = EMB + 50mg/kg TE group; E = EMB + 100mg/kg TE group.

The arrow pointing at V = Vacoulation in the optic nerve.

### 4.3.2 Effect of *Lantana trifolium* Ethanolic Extract on Ethambutol Induced Optic Nerve Axonal Demyelination

Demyelination was not observed in the optic nerve axons of the negative control group. However varying degrees of demyelination were observed in the remaining groups. Axonal demyelination was severe in the axons of the optic nerves of the positive control group and; the group treated with EMB and 25mg/kg of TE. Axonal demyelination was mild in the groups treated with EMB and 50mg/kg of TE and; EMB and 100mg/kg of TE.

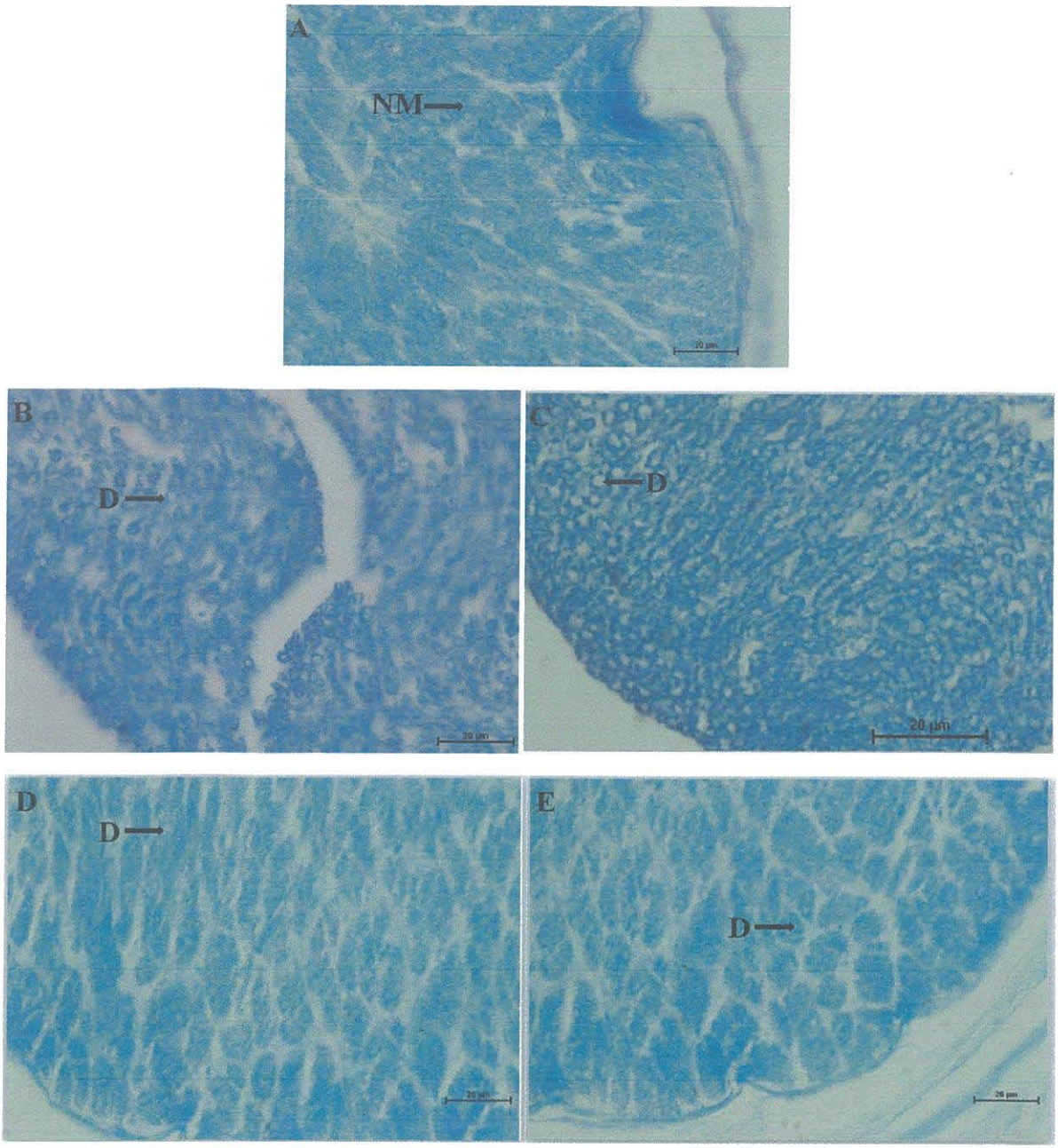



**Figure 9: Photomicrographs of Optic Nerve Cross Sections Showing Axonal Demyelination. Luxol Fast Blue Stain, X40.**

KEY: Image, A = negative control group; B = positive control group; C = EMB + 25mg/kg TE group; D = EMB + 50mg/kg TE group; E = EMB + 100mg/kg TE group. The arrow pointing at NM = Normal Myelination; D = Demyelination.

# CHAPTER FIVE
# DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

## 5.1 DISCUSSION

### 5.1.1 Effect of *Lantana trifolium* Ethanolic Extract on Ethambutol Induced Changes in Visual Acuity

*Lantana trifolium* had a dose dependent protection against visual acuity changes induced by Ethambutol (EMB). This was shown by a reduction in the escape latencies using a water maze. There was no significant difference in visual acuity in the 1st week probably due to delayed effects of EMB. During the 3rd week, there was improvement in escape latency possibly due to delayed effect of EMB and learning. This observation is similar to earlier findings (Baldi *et al.,* 2005; Gulinello *et al.,* 2009; Dhingra and Kumar, 2012), who reported a significant reduction of escape latency in water maze trained rats.

The group differences in the third week were not significant *($P > 0.05$)* when compared individually with those in the negative and positive control groups. This is possibly because the onset of symptoms of EMB induced changes is usually delayed (Garg and Kumar, 2008). Previous studies observed that structural injury to the optic nerve occurs earlier than visual function impairment (Pan *et al.,* 2013). The increase in escape latencies of the positive control group and the group treated with EMB and 25mg/kg of *trifolium* Extract (TE) during the fifth week could be due to a decrease in visual acuity as a result of EMB induced optic neuropathy. Similar observations have been reported about EMB toxicity (Heng *et al.,* 1999; Talbert Estlin and Sadun, 2010 ; Pan *et al.,* 2013), who observed that EMB induces changes in the optic nerve that manifest as a decrease in visual acuity. There was a further decrease in the escape latencies of the groups treated with EMB and 50, and 100mg/kg of TE during the fifth week, showing that *Lantana trifolium* ethanolic extract probably prevented EMB induced changes in the visual acuity.

Since the decrease in visual acuity is in part as a result of damage to the central fibers of the optic nerve which are the most vulnerable to EMB (Tawse *et al.,* 2014), we stipulate that *Lantana trifolium* ethanolic extract significantly prevented EMB induced decrease in visual acuity perhaps by protecting the optic nerve structure against EMB toxicity. This observation could be the reason for its tradition use in providing relief to some eye conditions with unknown causes of loss of visual acuity (Eldaly *et al.,* 2014).

### 5.1.2 Effect of *Lantana trifolium* Ethanolic Extract on Ethambutol Induced Changes on the Gross Morphology of the Optic Nerve

There were no significant gross morphologic changes induced by EMB observed in all experimental animals. The optic nerves of all the groups had a normal pinkish white color. Similar finding were reported previously by Kinoshita *et al.,* (2012) and Salgado-Moran *et al.,* (2013), showing that EMB toxicity did not have an effect on the color of optic nerve in experimental animals. However pale optic disc has been reported in patients with EMB induced optic neuropathy (Masvidal *et al.,* 2010).

The optic nerve color is due to the myelin layer surrounding the optic nerve axons which is white and the blood in the vessels of the covering meningeal sheath that give it a pinkish appearance (Spees *et al.,* 2013). Color change of the optic nerve has been reported in conditions that cause widespread myelin and axonal loss (Maresca *et al.,* 2013). The possible explanation for no change in the color is that probably more duration of exposure to EMB is required to have an extensive optic nerve demyelination and axonal loss.

### 5.1.3 Effect of *Lantana trifolium* Extract on Ethambutol Induced Changes in the Histology of the Optic Nerve

*Latana trifolium* extract had a neuroprotective potential against EMB induced changes in the histology of the optic nerve. The extract showed a dose dependent protection against optic nerve vacuolation and axonal demyelination. In this study, significant differences in the number of vacuoles were observed. The highest number (37.4±1.54) was observed in the positive control group that received EMB alone; this result is consistent with earlier observations (Chen and Liang, 1999; Chung *et al.,* 2009; Neuropathy *et al.,* 2016). They reported that EMB induced optic neuropathy causes vacuolar changes in the optic nerve. No significant $(P > 0.05)$ difference was found between the number of vacuoles in the positive control group and the group treated with EMB and 25mg/kg of TE. However, there was significant difference $(P<0.05)$ when the number of vacuoles in the groups treated with EMB and 50, and 100mg/kg of TE were compared to those of the positive control (EMB only) group.

Vacuolations show axonal degeneration induced by EMB (Zubair *et al.,* 2009). The exact mechanism how these vacuoles are formed has not been confirmed (Thee *et al.,* 2007; Fonkem *et al.,* 2013). However the suggested mechanism is related to the chelating effects of EMB and its metabolites that interfere with several mitochondrial metal containing enzymes (Guillet *et al.,*

2010b), such as the iron containing complex I and copper containing complex IV (Kinoshita et al., 2012). This interrupts oxidative phosphorylation and mitochondrial function leading to adenosine triphosphate (ATP) depletion (Wang *et al.,* 2012), generation and accumulation of reactive oxygen species (ROS) that cause oxidative stress in the optic nerve (Areti *et al.,* 2014). The resultant effect is anaerobic glycolysis, forming lipids inside the axon that microscopically appear like vacuoles (Chen *et al.,* 2015). It is speculated that *Lantana trifolium* protected the optic nerve against EMB induced vacuolation by interfering with this mechanism.

The protective potential of *Lantana trifolium* against EMB induced optic nerve vacuolations is probably as result of its anti-oxidative activity which is attributed to its high amounts of phenols (Salabarría *et al.,* 2009). This is done through blocking the formation of ROS and scavenging them once they have been formed by donating electrons to the highly reactive free radicals (Perumal *et al.,* 2012) . This results into formation of stable compounds and thus reduction of oxidative stress in the optic nerves. In another study, caffeic acid phenethyl ester, a phenolic compound found in plants was found to significantly decrease vacuolation by preventing oxidative stress in the optic nerve of EMB treated rats (Uzar *et al.,* 2014).

Ethambutol induced severe demyelination in the axons as observed in the positive control (EMB only) group and the group treated with EMB and 25mg/kg of TE. The result of our investigation is consistent with the findings of (Kozak *et al.,* 1998; Heng *et al.,* 1999; Han *et al.,* 2015). However, Zubair *et al.,* (2009) reported differing results, they did not observe demyelination of the axons after four weeks of EMB treatment. This difference could be as a result of 1 week difference in the duration of the experiments. Demyelination in the groups treated with EMB and 50, and 100mg/kg of TE was mild; suggesting that *Lantana trifolium* extract effectively protected the optic nerve against EMB induced axonal demyelination at doses of 50mg/kg and 100mg/kg.

Ethambutol toxicity damages myelin, a fatty acid layer produced by the oligodendrocytes that surrounds optic nerve axons (Fancy *et al.,* 2011). Myelin is responsible for fast transmission of signals through the nerves (Crawford *et al.,* 2014). Damage to the myelin sheath results into progressive axonal loss (Ali *et al.,* 2012). A similar mechanism as described above of EMB metal chelating effect resulting into oxidative phosphorylation disruption and mitochondrial dysfunction has been suggested as well for its induced axonal demyelination (Guillet *et al.,*

2010). Other studies reported that EMB toxicity induces demyelination through inhibition of phosphorylation by cyclic adenosine monophosphate (AMP) dependent protein kinase-A (Griffith *et al.,* 2005). This is the same mechanism for phosphorylating myelin basic proteins (Chan and Kwok, 2006). By compromising respiratory function, a major consequence of reactive oxygen species accumulation may generate some compensatory proliferation of mitochondria in the endothelial and smooth muscle cells of optic nerve blood vessels (Osaguona *et al.,* 2014). These changes result into vascular inflammation and thus a reduced blood supply to the nerve tissue that precedes myelin breakdown (Rasool *et al.,* 2015).

Since oligodendrocytes are preserved in the early stages of demyelination (Kim *et al.,* 2012), the myelin sheaths around the optic nerve axons can be reconstructed after eliminating the cause of the myelin damage (Young *et al.,* 2013). Therefore it is possible that *Lantana trifolium* ethanolic extract protected the optic nerve against EMB induced demyelination by restoring oxidative phosphorylation and inhibiting inflammation. The plant's anti-oxidative activity has already been mentioned in previous studies (Imbenzi et al., 2014). *Lantana trifolium* ethanolic extract is known for its anti-inflammatory activity attributed to the high flavonoid content (Silva *et al.,* 2005). The mechanism of how it prevents inflammation has not been studied sufficiently. However Julio *et al.,* (2010) reported that *Lantana trifolium* inhibits inflammation by reducing histamine release .

## 5.2 CONCLUSIONS

*Lantana trifolium* had a dose dependent neuroprotective potential against ethambutol induced visual acuity changes. A post-HOC Tuckey test showed a significant decrease in visual acuity in the group that received ethambutol only when compared against the groups that received ethambutol and *trifolium* extract. Therefore the alternative hypothesis was accepted. This study stipulates that this effect was due to *Lantana trifolium* ethanolic extract protecting the optic nerve structure against EMB toxicity.

No significant gross morphologic changes were observed in the optic nerves of all experimental animals. Thus the null hypothesis was accepted and the alternative hypothesis was rejected.

*Lantana trifolium* ethanolic extract had a dose dependent neuroprotective potential against ethambutol induced changes in the histology of the optic nerve. A post-HOC Tuckey test showed a significant difference in the optic nerve histology of the group that received ethambutol only when compared against the groups that received ethambutol and *trifolium* extract. The potential of *Lantana trifolium* ethanolic extract to protect optic nerve against EMB induced changes could be as a result of its anti-oxidative and anti-inflammatory activities.

## 5.3 RECOMMENDATIONS

The effects of *Lantana trifolium* extract on ethambutol induced optic neuropathy in humans should be studied since the plant is already traditionally used to treat some eye conditions. In this study a modified Morris water maze was used to determine visual acuity, however we suggest that studies using more sensitive tests to measure visual acuity in Wistar rats should be carried out. Luxol fast blue, hematoxlyin and eosin staining techniques were used to evaluate EMB induced histological changes in the optic nerve. More studies using immunohistochemical techniques should be performed. The exact phytochemical component and mechanism of action of *Lantana trifolium* responsible for its neuroprotective potential against EMB induced changes were not determined. Therefore we recommend that further studies to establish the exact phytochemical component and mechanism of action are conducted.

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## INSTITUTIONAL REVIEW AND ETHICS COMMITTEE (IREC)

**October 04, 2016**

OWEMBABAZI ELNA
MSc.ANA/0003/142/DU

# LETTER OF APPROVAL

This is to certify that the research proposal titled **"Protective Potential of *Lantana Trifolia* Extract against Ethambutol Induced Optic Neuropathy"** was reviewed by the Research Subcommittee of the Board of Postgraduate Studies and Research Directorate of Kampala International University-Western Campus (KIU-WC) in its meeting on August 16th, 2016 for its Scientific Validity and Ethical appropriateness and was approved subject to minor corrections.

This proposal was finally approved on October 03, 2016 after the expedited review following the minor corrections. You may now start conducting your research.

The Research Subcommittee retains the powers to continue monitoring how you are conducting the research.

Signed by:

Monicah for Dr. Medard Twinamatsiko

**Assoc.Director, Research Innovation Extensions and Publications**

KIU WESTERN CAMPUS
04 OCT 2016
P. O. BOX 71
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School Of Post Graduate Studies & Research

**Date/Stamp**