

# *Lantana Trifolium* Ethanolic Extract has a Protective Potential against Ethambutol Induced Changes in Visual Acuity

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## Abstract

**Introduction:** Ethambutol (EMB), an important drug in treatment of multidrug resistant tuberculosis, has been associated with severe side effects including visual impairment.

**Purpose:** To establish the protective potential of *Lantana trifolium* ethanolic extract against EMB induced changes in visual acuity.

**Materials and methods:** Experimental design involving 25 male adult Wistar rats of 110-130g average weight, divided into five groups each comprising five animals. 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 for five weeks. Visual acuity was determined by the mean escape latencies obtained using a modified Morris water maze.

**Results:** *Lantana trifolium* ethanolic extract had a dose dependent protective potential against EMB induced changes in visual acuity. This was shown by the significant increase in the escape latencies of positive control group when compared with those of the group A (4.35±0.50), D (4.85±0.65), and E (3.6±0.38). This effect is likely due to the ability of *Lantana trifolium* to prevent inflammation and accumulation of anti-oxidants in the optic nerve.

**Conclusion:** *Lantana trifolium* ethanolic extract has a dose dependent protective potential against EMB induced changes in visual acuity. Studies to determine the exact phytochemical component and mechanism of action responsible for this effect should be conducted.

## Introduction

Ethambutol (EMB) is an antibiotic belonging to the amino-alcohol group [1]. It is an efficacious first line antituberculosis agent [2]. EMB is known to cause changes in the optic nerve that result into toxic optic neuropathy (TON) [3]. This was established soon after its discovery in 1960s. In the early 1960's, studies reported EMB induced optic neuropathy in 50% of patients receiving 60–100 mg/kg/d of EMB [4]. This toxicity is generally described as dose and duration dependent [3], but there is no effective dose that is entirely free from the threat of toxicity [5]. The toxicity is observed at a dose as low as 12.3 mg/kg [6]. 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 [7], occurring at least 1.5 months after therapy initiation [8]. A number of cases of toxicity occurring as early as after less than six days of treatment have been reported [9].

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 [10]. Although EMB induced changes are often reversible when EMB is discontinued, in some patients recovery is incomplete [11,12]. Moreover, permanent visual impairment has also been reported in some patients [11]. In Uganda TB burden is still high [13], 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 [14]. Therefore, means of protecting the optic nerve against EMB toxicity need to be sought.

Traditional eye medicines (TEM) are commonly used to treat different eye diseases worldwide [15]. *Lantana trifolium* three leafed, scrambling, evergreen shrub (Figure 1), is widely used to treat many eye conditions such as blindness, glaucoma, conjunctivitis, and bacterial and viral eye infection [16]. It is also used to treat epilepsy, infant cerebral malaria, asthma, sinusitis, menstrual pains,

mental illness, sickle cell anaemia, stomach ache and skin conditions [17]. Phytochemical composition of *Lantana trifolium* has not been studied extensively. However previous studies have reported that the plant contains phenols, phenylpropanoids, flavonoids and terpenoids [18]. Previous ethno-medicinal studies about this plant have reported that its extract inhibited carrageenan and histamine induced rat paw edema [19]. The anti-inflammatory effect of extract is reported to be possibly due to histamine reduction [15]. Another study reported that *Lantana trifolium* extract has anti-oxidative activity due to the high amounts of phenols it contains [20]. 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 [21], forming stable compounds and thus reducing oxidative stress in the optic nerves. This study established if *Lantana trifolium* ethanolic extract could protect against ethambutol induced changes in the visual acuity.

## Materials and Methods

Twenty five male adult wistar rats of 110-130g average weight were used in this experiment. These were housed in cages at room temperature ( $25^{\circ}\text{C} \pm 1$ ), exposed to 12 hours of dark and light cycles. The animals were fed on commercial rat pellets and provided with tap water ad libitum.

The animal grouping, plant material collection, preparation of the extract, administration of ethambutol and *Lantana trifolium* extract were performed as previously described [22].

Ethambutol at doses of 100mg/kg/day induces visual impairment in different experimental animals.

Dose given =  $\frac{\text{dose (mg/kg)} \times \text{animal weight (grams)}}{1000}$  [23].

Concentration (mg/ml) x 1000

## Ethical considerations

Directorate of Postgraduate Studies and Research of Kampala International University Western Campus approved this study to be carried out. All animal studies in this experiment were conducted in accordance with the 2010 National Research Council Guide for Care and use of Laboratory Animals. The number of animals used

was minimum and inflicting pain to the animals was minimized by sacrificing them under anaesthesia.

## Visual acuity testing

A visible platform Morris water maze was used to determine the visual acuity (Figure 2). 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  $25^{\circ}\text{C}$  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 quadrants. 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 [24].

## Data analysis

Using graph pad prisms V6, mean  $\pm$  SEM were obtained and 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 which group means differed, considering a p-value of  $< 0.05$  to be statistically significant.

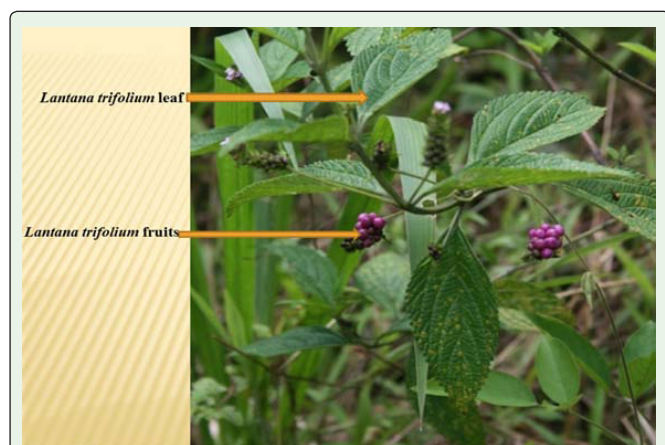


Figure 1: Lantana trifolium Plant.



Figure 2: A modified Morris water maze.

**Table 1:** Mean Escape Latency.

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

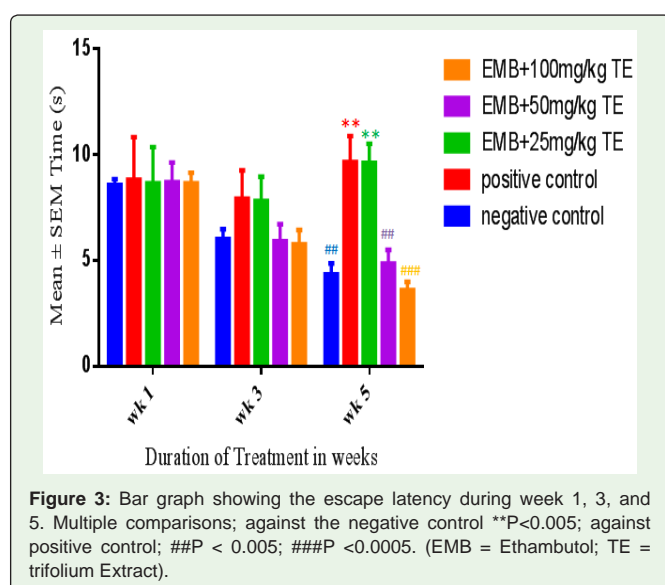
N = number of rats in each group; EMB = Ethambutol; TE = *Trifolium* Extract. Multiple comparisons; against the negative control group <sup>\*\*</sup>P<0.005; Against positive control group, <sup>##</sup>P < 0.005, <sup>###</sup>P <0.0005.

## Results

In all the groups the escape latencies obtained during the first week were not significantly different ( $P > 0.05$ ) revealing no significant change in the visual acuity? 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 and the group that received EMB+ 25mg/kg *Trifolium* Extract (TE) increased (9.65±1.22) and (9.6±0.90) respectively (Table 1). 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 that received EMB+ 50mg/kg TE, but the difference was more significant in the group that received EMB+ 100mg/kg TE.

## Discussion

*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 (Figure 3). There was no significant difference in visual acuity in the 1st week in all



the groups probably due to delayed effects of EMB. During the 3rd week, all the groups showed a reduction in the escape latency possibly due to delayed effect of EMB and learning. This observation is similar to earlier findings [25-27], 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 [28]. Previous studies observed that structural injury to the optic nerve occurs earlier than visual impairment [29]. The increase in escape latencies of the positive control and the group that received EMB+ 25mg/kg *Trifolium* Extract (TE) during the fifth week could be due to a decrease in visual acuity as a result of EMB induced visual impairment. Similar observations have been reported about EMB toxicity [3,30,31], 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 group that received EMB+ 50mg/kg TE and the group that received EMB+ 100mg/kg 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 [32], 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 [33]. In our previous study we observed that *Lantana trifolium* ethanolic extract significantly reduced EMB induced axonal demyelination and vacuolation [22]. Thus, *Lantana trifolium* protected the optic nerve structure against EMB toxicity, through its anti-oxidative and anti-inflammatory activity reported in experimental animals [34].

## Conclusions and Recommendations

*Lantana trifolium* ethanolic extract had a dose dependent protective potential against ethambutol (EMB) induced changes in visual acuity. This effect of *Lantana trifolium* ethanolic extract could be as a result of its ability to protect the optic nerve structure against inflammation and anti-oxidant accumulation. We suggest that studies using more sensitive tests to measure visual acuity in Wistar rats are carried out. Further studies to establish *Lantana trifolium* exact phytochemical component and mechanism of action responsible for its protective potential should be conducted.

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