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NEUROTOXICOLOGICAL EVALUATION OF PLUMBAGO *ZEYLANICA* L. ROOT EXTRACT AND AMELIORATIVE EFFECT OF VITAMIN.E IN AN EXPERIMENTAL MICE

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ABSTRACT
Plumbago zeylanica is a medicinal plant with various therapeutic application since time immemorial, in the present study adverse brain toxicity of this vital medicinal plant was evaluated in biochemical parameters. The significant pathological changed in histological study were also noted in *Plumbago zeylanica* plant extract treated mice brain. alteration in biochemical parameters was noted with significant alteration level of LPO and GOT, GPT activity which may be due to plant extract admistration. The elevation in cerebral cortex was significant ($p<.01$) in *Plumbago zeylanica* treaeted mice and mid brain tissue of *Plumbago zeylanica* also showed significant($p<.05$) elevation in all the biochemical parameters in plant extract treated group. In the present study Pretreatment of antioxidant vit.E with plant extract group showed recovery from stress when compared to plant extract treated alone. The cerebral cortex and mid brain tissue of pretreatment with Vit.E group showed highly significant in the present study.

Keywords: VitaminE, Plumbago zeylanica, Neurotoxicity, LPO, GOT, GPT, neuroprotection.

1. INTRODUCTION
The brain is an important part of the organism functioning as coordinating and regulating system, it is an extreme heterogeneous with a large number of different neuronal and non neuronal cell types with extensive morphological differentiation within the cell (Raner *et al*.,2002). It is deficient in oxidative defense mechanisms and hence it is considered highly vulnerable to oxidative damage than other organs of the body due to its high oxygen consumption, presence of high levels of polyunsaturated fatty acids and low levels of antioxidant and nonproliferative nature of neurons, which may lead to various neurodegenerative diseases (Floyd and Carney, 1992).
Medicinal plants are the local heritage with global importance, herbal remedies have become popular over the past decade and they are widely used for the treatment and prevention of various diseases. *Plumbago zeylanica* Linn. (Plumbaginaceae) is one of the well-known Ayurvedic drug. From the previous work of many authors numerous studies have shown that the root have anti-fertility activity (Sandeep *et al*,2011),antimicrobial activity (Jetty *et al*, 2010), antifungal(Mehmood *et al*, 1999), antiinflammatory (Oyedapo,1996), antibacterial (Jeyachandran *et al*, 2009), anticancer (Melo *et al*,1974). It is also reported to have actions in the central nervous system including stimulatory (Bopaiah and Pradhan,2001). The roots was also reported to be a powerful poison when given orally or applied to ostium uteri and causes abortion (Azad Choudhary *et al*.,1982). Herbal drugs, though natural, can

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still cause serious adverse effects on the body, ranging from cancer to dysfunctions of vital organs such as liver, heart, lung etc and even death (Aschwanden, 2001).In view of earlier work reported by several authors, it is thus necessary to expand the present study for evaluation of neurotoxicological effects in mice brain. Vitamin E believed to be a predominant chain breaking lipid-soluble antioxidant is the primary free radical scavenger and prevent lipid peroxidation (Cerolini *et al*., 2000). Therefore, the present study is carried out to investigate behavior activity, the alteration in biochemical parameters and histopathological changes in mice brain with long term admistration of *Plumbago zeylanica* root extract and to evaluate neuroprotection of antioxidant vitamin.E with *Plumbago zeylanica* root extract admistration in intraperitoneal mode.

2. MATERIALS AND METHODS

2.1 Chemicals

VitaminE (DL-α-tocopherol, liquid) [(C29H50O2] was obtained from Himedia Laboratories Pvt. Ltd, Mumbai. All other chemicals used in the present experiment were of analytical grade.

2.2 Preparation of plant material

The roots of *Plumbago zeylanica* was collected from Ahthibung local forest reserved and leaves were collected for taxonomic identification. The plant was identified and authenticated from Botanical survey of India, Shillong. The collected roots were cleaned by washing in running water and then it was cut into pieces and air dried in cold shaded for 20-25 days. The roots were then grinded to powdered form with electrical grinder and was keep in airtight container for further use. In the present study 500g of extract powder was soak in 80% methanol for 4days with occasional stirring with glass rod and at the end of 4^{th} days it was filtered with watt-man filter paper with the help of funnel and the filtered was tested for biochemical components then the filtered was heated at low intensity to obtained in semidry form and again biochemical components integrity was tested to see if the chemical components remain same even after heating to semi dry with low intensity .

2.3 Collection and Maintenance of Animal:

Healthy male albino mice weighing 25±3 gm b.w were collected from Pastuer Institute Shillong for the experiment. The animals were kept in cages under normal laboratory conditions in 12h light and 12h dark periods. The animals were fed on standard balanced diet. The water was made available ad libitum to the animals. Experimental animals were handled according to the guide lines of Assam University Ethical Committee and supervision of experiments on animal. The LD50 was calculated by following method of Lorke(1983). The animals were observed for manifestation of physical sign of toxicity and number of death within 24 hrs. The LD50 was calculated as the geometric mean of the maximum dose producing 0% mortality and minimum dose producing 100% mortality, and LD50 of 80% methanol root extract of *Plumbago zeylanica* was determined in i.p route.

2.4 Experimental design

The mice were divided into four (4) groups with minimum of five (n=5) animals in each group. The admistration of respective dosing was done regularly between 8 am to 9am with respective group, in group vi, Vit.E admistration was done 60min. prior to Plumbago zeylanica extracts.

Group I :Control (distilled water/ normal saline).

Group II : Plumbago zeylanica (160 mg/kg b.w. i.p).

Group III: Antioxidant dose (Vit.E 50 mg/kg b.w. i.p)

Group IV: Antioxidant plus Plumbago zeylanica treated group (given Vit.E 50mg/kg b.w,i.p plus Plumbago zeylanica (160mg/kg b.w, i.p).

After 15 days of treatment a behavior paradigm and biochemical parameters study was done.

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2.5 Behaviour study test.

2.5.1 Elevated plus-maze test. (EPZ).

Anxiety in rodents was assessed using the elevated plus maze (Pellow et al,1985:Lister, 1987).The apparatus consisted of two open arms (35×5 cm) and two closed arms (35×5×20 cm) in perpendicular position, extending from a central platform (5×5 cm).The maze was elevated 45 cm from the floor. After treatment, the animal was placed at the center of the plus maze, facing one of the open arms, during the 5min test the number of entries in open and close arm and the time spent on open arms was measured by direct observation. Entry into an arm was defined as the point when the animal places all four paws onto the arm. The maze was then cleaned with a solution of 70% ethyl alcohol and permitted to dry between tests.

2.6 Biochemical parameters.

After completion of behavior study the animals were sacrificed by cervical dislocation for biochemical study. The whole brain was immediately dissected out, washed in normal ice cold saline and immediately cerebral cortex and midbrain was separated and processed for various biochemical estimation.

2.7 Determination of LPO.

Lipid peroxidation (LPO) levels in the three brain regions were determined by the method of Okhawa et al.1979. According to this method, 15 gm trichloro acetic acid was added to 100 mL of 0.25 N hydrochloric acid. 15 mg of thio barbituric acid was dissolved in 4 mL of trichloro acetic acid in HCl mixture. To 0.1 mL of homogenate, 0.4 mL trichloro acetic acid–thio barbituric acid–hydrochloric acid mixture was added and kept in a boiling water bath for 20min. Then, it was cooled to room temperature gradually, followed by addition of 1 mL of n-butanol, vortexed well and centrifuged for 10 min. The supernatant was taken and was read at 532 nm in spectrophotometer. The level of LPO was expressed as nM of MDA (an intermediary product of lipid peroxidation, using thio barbituric acid)/mg protein.

2.8 Determination of GOT & GPT activity.

Glutamate oxaloacetate transaminase (GOT) and Glutamate pyruvate transaminase (GPT) activity were determined by the method of Reitman and Frankel (1957). Homogenate (50 mg/ml, w/v) was prepared in 0.25 M cold sucrose solution. Optical density was measured at 546 nm. The enzyme activity was expressed as µmoles of pyruvate formed/mg protein.

2.9 Protein Estimation.

The concentration of protein in the brain homogenates were evaluated using the Lowry method (Lowry et al., 1951).

2.9.1 Histopathological study:

Histopathological study was done according to (Bancroft *et al*,1996) method. Tissue from mid brain and cerebral cortex were separated and fixed in 10 per cent formalin and were processed by conventional method, embedded in paraffin, sectioned at 4-5 µm and stained by haematoxylin and eosin. Tissues were examined under a light microscope. Histopathological study was carried out in the department of Pathology, Silchar Medical college and hospital (SMCH).

2.9.2 Statistical analysis:

The results of all data are expressed as mean ± S.D. for five mice in each group. Differences between groups were evaluated by one way ANOVA followed by LSD multiple comparisons test (*P<0.05 & **P≤0.01 vs Control) using the SPSS software package for Windows.

2.10 Results

The median lethal dose (LD50) in the present study in methanol root extract of Plumbago zeylanica was 750 mg/kg i.p in the experimental mice.

2.10.1 Histological - Histopathological observations in the mice brain tissue

In the present study, the untreated/control brain tissue shows the normal histoarchitecture of nerve cells. In *Plumbago zeylanica* (160 mg/kg b.w) treated group, the brain tissue shows the damaged nerve cells in some area with irregular cell distribution with deformed cellular shape and size and necrosis were noticed compared to control. However, pretreatment with antioxidant vitamin.E (50 mg/kg b.w i.p) with *Plumbago zeylanica* extract (160mg/kg b.w i.p) shows remarkable ameliorated histoarchitecture in the brain

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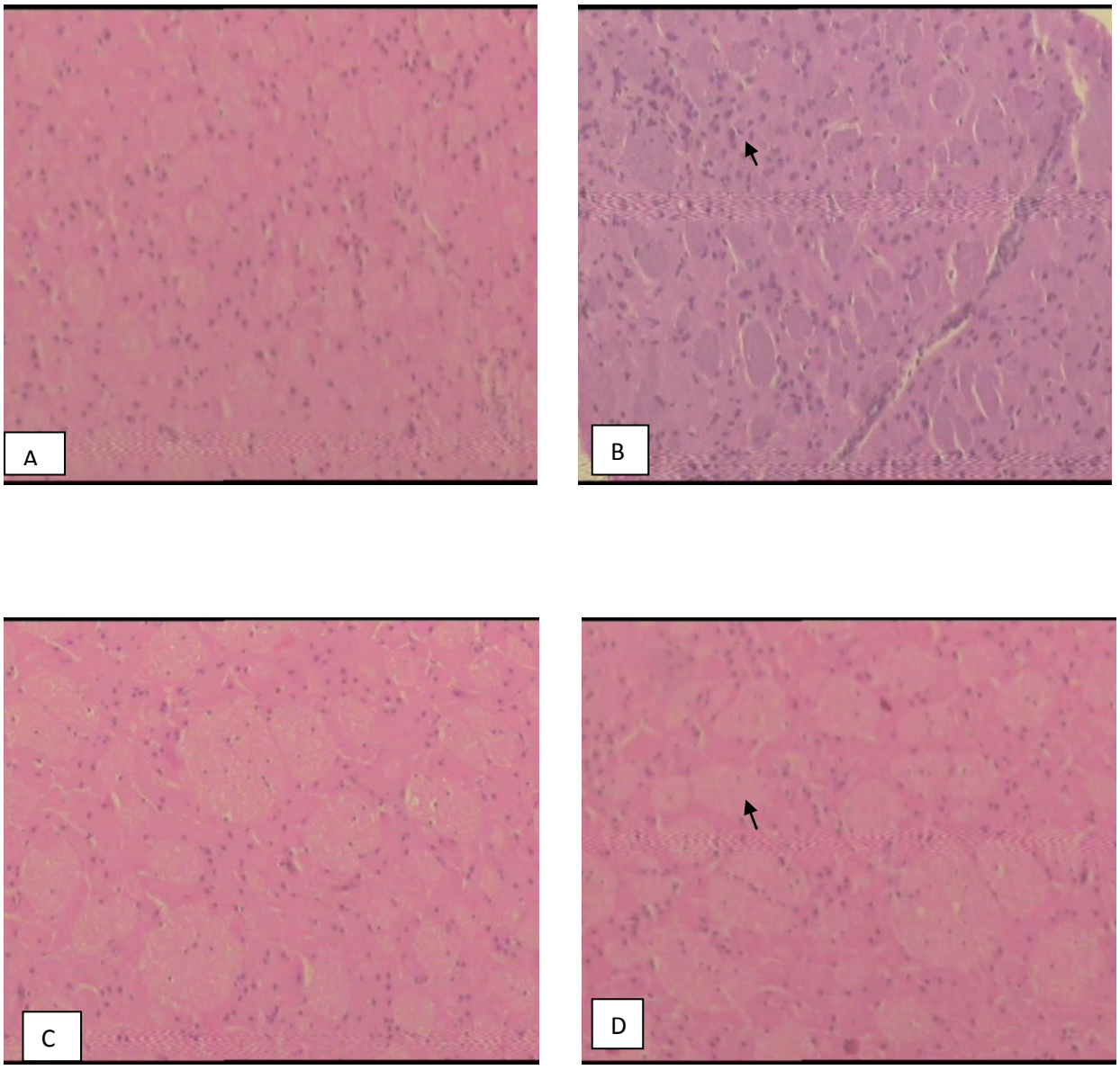
with normal neuronal architecture and nerve cells and absence of necrosis as shown in fig1&2 in the mid brain and in cerebral cortex of mice brain.



Figure,1. Microscopic study of Mid brain. (A) Control group with normal brain architecture. (B) Neuronal degeneration, necrosis and obliterated shape of neurons in mid brain shown in (↑) of male albino mice administrated with *Plumbago zeylanica* extract for 15 days. (C) showing normal with vit.E admistration. (D) Brain of mice administrated with vit.E. (50 mg/kg b.w)+*Plumbago zeylanica*(160mg/kg) for 15days showing protection of neuronal degeneration, necrosis and to normal recovery from obliterated shape with absence of necrosis in mid brain shown in (↑) tissue of male albino mice. H&E X100.

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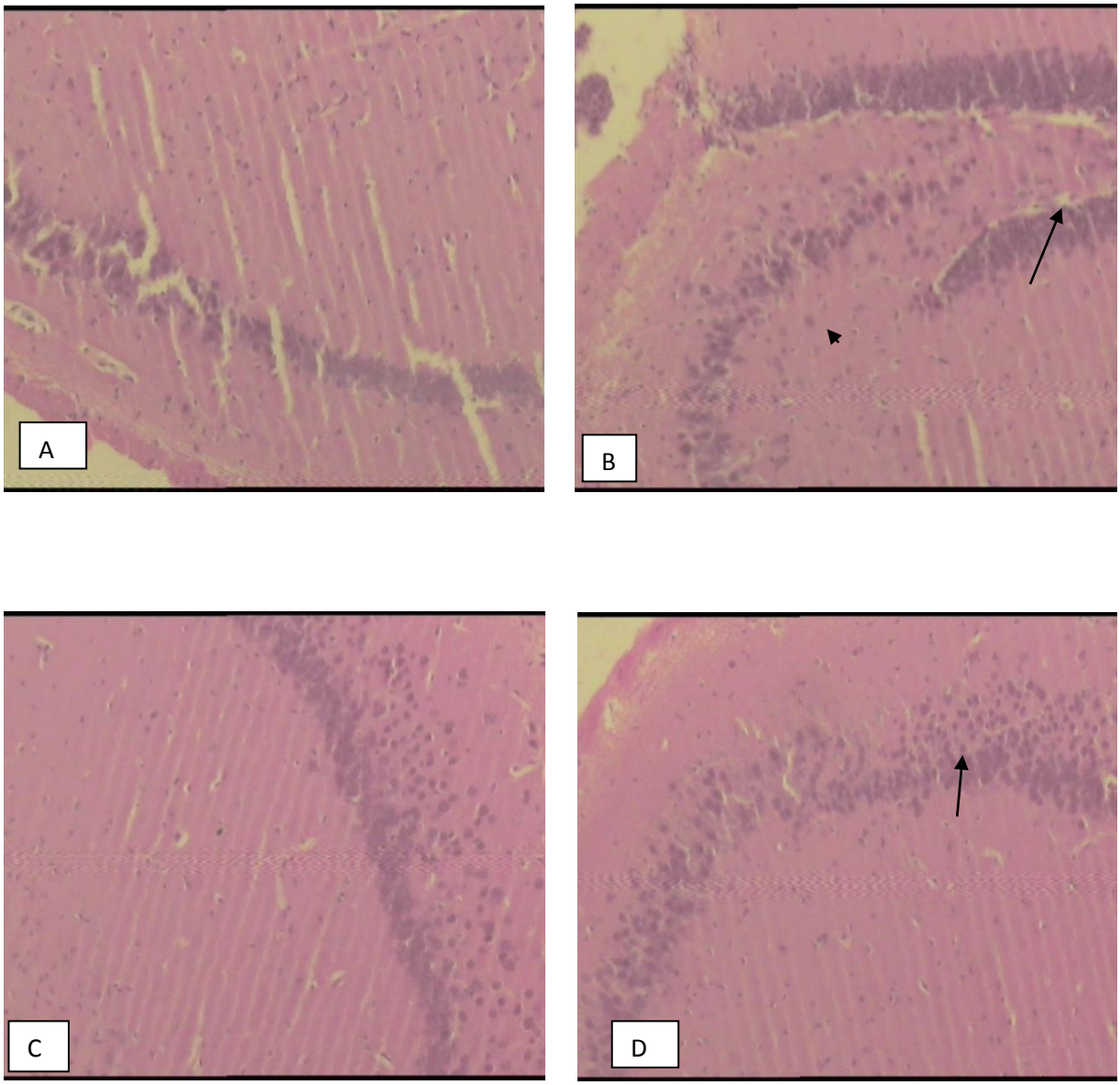

Figure.2. Microscopic study of cerebral cortex.(A) Control brain of mice. (B) Brain of mice administrated *Plumbago zeylanica* for 15days showing massive diffuse cells as well as necrosis shown in arrow (↑) in cerebral cortex tissue.(C) Brain of mice administrated with vit.E. (D) Brain of mice administrated with vit.E. (50mg/kg b.w) + *Plumbago zeylanica* (160 mg/kg) for 15 days showing protection and recovery from massive diffuse cells and necrosis in cerebral cortex. H&E X 40.

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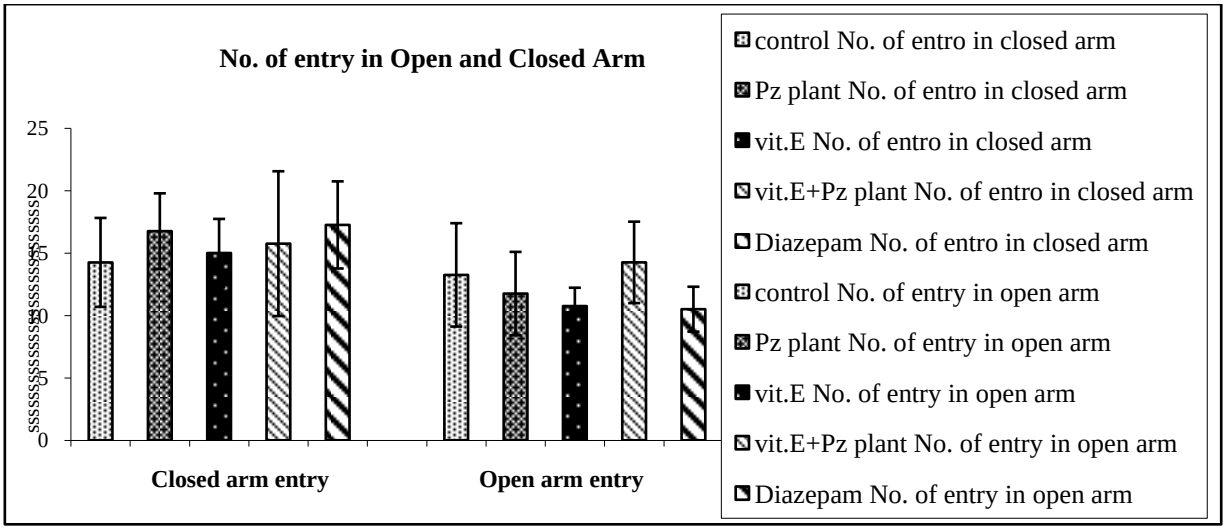

Figure.3. The effects *of Plumbago zeylanica* methanol root extract (160 mg/kg i.p.) and diazepam (DZP 2 mg/kg i.p.) on the number of entries in the open arm and closed arm and the time spent in open arms of the elevated plus-maze in mice with pretreatment of Vit.E. Data represent means±S.D of five animals in each group during the 5-min test session. Comparisons were made by using a one-way ANOVA followed by LSD multiple Comparison test: $*p < 0.05$ compared with control group.

Figure.3 shows Anxiety studies using elevated plus maze with a mild anxiety in mice admistered with *Plumbago zeylanica* extract (160 mg/kg), there was no significant entry to the open and closed arm compared with those of the control groups but time spent in the closed arms was significantly (p<.05) more than time spent in open arms, data not shown. On the other hand, mice pretreated with antioxidant VitaminE showed a significant(p<.05) increased time spent in the open arms compared with those of the other groups. There were no significant alterations in the number of entries in the closed arms or in the total number of entries in arms between the experimental groups.

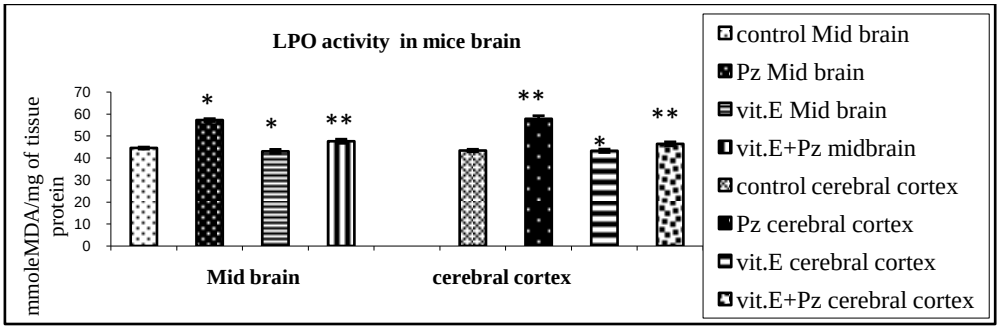

Fig.4. Effect on LPO activity in cerebral cortex and mid brain regions following methanol root extract of *Plumbago zeylanica* (160 mg/mg i.p) administration with pretreatment of Vit.E(50 mg/kg). Each column represent mean±S.D (n=5). Comparisons were made by using one way ANOVA followed by LSD Multiple Comparison test (* P<0.05; ** p< 0.01, vs control).

LPO level increased significantly in *Plumbago zeylanica* treated group (P < 0.01) in cerebral cortex and (p< .05) in mid brain as compared to control group. Pretreatment of antioxiant vit.E with plant extract treated group significantly altered the LPO level. Lipid peroxidation was significantly(p< .01) decline in cerebral cortex tissue as shown in fig4. also mid brain tissue showed significant declined at (p< .05) in VitE pretreated mice compared to Plant extract treated group.

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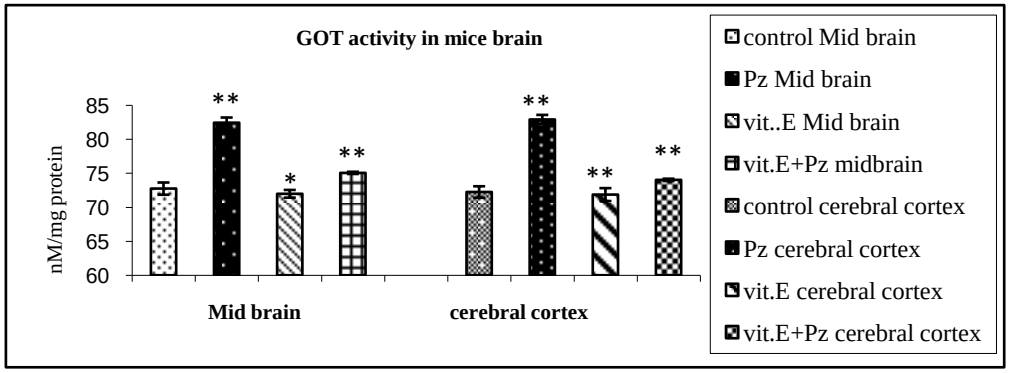

Fig.5. Effect on GOT activity in cerebral cortex and mid brain regions following methanol root extract of *Plumbago zeylanica* extract administration and pretreatment of Vit.E (160 mg/kg) with plant extract treated group. Each column represent mean±S.D (n=5). Comparisons were made by using one way ANOVA followed by LSD Multiple Comparison test (* P<0.05; ** p< 0.01, vs control).

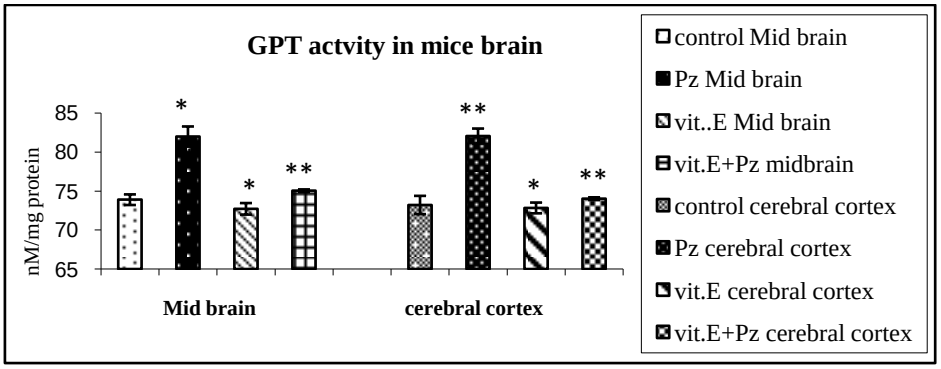

Fig.6. Effect on GPT activity in cerebral cortex and mid brain regions following methanol root extract of *Plumbago zeylanica* administration with Vit.E pretreated group. Each column represent mean±S.D (n=5). Comparisons were made by using one way ANOVA followed by LSD Multiple Comparison test (* P<0.05; ** p< 0.01, vs control).

The effect of GOT and GPT in treated mice brain was well pronounced and the cerebral cortex showed significant elevation(p< .01) of GOT and GPT activities in *Plumbago zeylanica* treated group similar observation with elevation in GOT and GPT activities in mid brain was found significant(p< .05) in plant extract treatment group. However, pretreatment of vit.E (50 mg/kg) with *Plumbago zeylanica* results in protection from the alteration in GOT and GPT activities compared to *Plumbago zeylanica* treated alone. As shown in fig,5&6, the GOT and GPT level was decreased to a certain extent closed to control.

2.11 Discussion

In the present study histopathological lesions were observed in mice brain by exposing to the methanol root extract of *Plumbago zeylanica* extract (160 mg/kg) at repeated dose during 15days exposure. Various pathological changes like necrosis and cellular degenerative with massive diffuse cells and changes have been observed in experimental mice brain in plant extract treated mice compared to control. The present investigation with severity of histopathological lesions indicates that the repeated exposure to plant extracts cause deleterious effects and making less fit for better survival. Our results are in consistent with similar to necrotic lesion observed following exposure to certain toxic xenobiotics such as ethanol, acetaminophen, carbon tetrachloride and bromobenzene (Dapar et al., 2007).Similar study on histopathological changes in animals by heavy metals have been reported earlier by several workers(Kumar and Pant, 1981; Akhilender Naidu, 1982; Usha Rani,1986). The present results are in agreement with the results of (Savory and Garruto 1998: Vogelbruch et al, 2000) with neuronal degeneration and neurodegenerative diseases associated

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with aluminum. The cerebral cortex are the key structures of memory formation. In the present study the brains tissue of experimental animal showed changes in histoarchetecture and necrosis in cerebral cortex and in mid brain compared to control which are form of neuro-degeneration. Similar result was reported by (Buraimoh, et al, 2011) with accumulation of Aluminium in the brain regions. Evidence of mild lesions in parts of the brain indicates the ability of the extract to cross the blood-brain barrier. In this study, pretreatment of antioxidant vitamin.E with plant extract, produced a reversal trend in most of the negative effects of behavior and showing protection in the brain tissue from pathological lesion with absence of necrosis and cellular. Similar result with protection of vitaminE were reported in behaviour (Uche et al., 2008; Sanchez et al., 1999). and in brain (Aschner et al., 2010).

Decreased open arm activities and increased risk assessment behaviours without concomitant changes in general locomotion and exploration indicate increased anxiety in the Elevated Plus-Maze (Cole & Rodgers, 1994; Lee & Rodgers, 1991). The index of open arm avoidance also gives a measure of anxiety (Trullas & Skolnick, 1993). Similarly in the present study significant ($p<.05$) lesser time entry and time spent in open arm was noted which is correlated with the earlier work. Where as, the evaluation of the putative antioxidant Vit.E in EPZ tests showed a significant increase in both the number of entries and time spent in the open arms of the maze compared to Plant extract treated alone which is similar to the effects observed after administration of the reference anxiolytic drug diazepam.

Lipid peroxidation is related with cellular injury and is commonly used as an indicator of oxidative damage in cells and tissues and it is regarded as one of the primary key events in cellular damage. The brain has been shown to be more vulnerable to injury by lipid peroxidation (Naffah Mazzacoratti et al., 2001).Elevation of LPO level in *Plumbago zeylanica* treated mice are in accordance with the findings of (Bharathi and Jagadeesan, 2012) with significant increased in LPO level in brain tissue of rat with mercuric chloride intoxicated rat. Similar result was reported by (Subhashini et al., 2011) with increased LPO in myocardial homogenate of Isoproterenol administered rats. With fluoride intoxication in rat brain (Shivarajashankara et al, 2001). Nascimento et al, (2005) also found elevated LPO when exposed to Pilocarpine. Glutamate oxaloacetate transaminase and Glutamate pyruvate transaminase (GOT and GPT) are measured as useful biomarkers to determine cellular impairment and cell rupture or biomarkers of tissue injury. GOT and GPT play a vital role in protein and carbohydrate metabolism and act as an indicator for tissue damage (Nemcsok et al., 1981; Nemcsok and Boross, 1982). In the present study elevated GOT and GPT level was noted in methanol root extract of Plumbago zeylenica in mid brain and cerebral cortex tissue of plants extract admistered mice compared to control. Similar study was reported by (Bakthavathsalam and Srinivasa Reddy, 1982) while studying the intoxication effects of lindane on the activities of GOT and GPT in different tissues of A testudineus, reported an elevated enzymes in different tissues studied.(Narasimha Murthy et al, 1985) also reported elevated GOT and GPT enzymes in brain, liver, muscle and gill of Oreochromis mossambicus exposed to lethal (0.15mg/ litre) and sub-lethal (0.05mg/ litre) concentrations of lindane. Also alteration with elevated GOT and GPT enzymes was reported in different tissues of Tilapia mossambica exposed to parathion-methyl (Rao and Rao 1983).

Antioxidants are known to reduce oxidative induced reaction. α tocopherol is an important antioxidant in biological systems. It inhibits peroxidation of membrane lipid by scavenging lipid peroxyl radical with formation of tocopheroxyl radical as a consequence (Arita *et al*., 1998). VitaminE is the major lipid soluble chain breaking antioxidant in the body tissues and effectively protects against neuronal damage (Badole et al, 2007: Bonnefont-Rousselot 2004). In the present study pretreatment of vit.E with methanol root extract of Plumbago zeylenica showed recovery/ameliorated from intoxication of plant extract admistration, as the number of entry in open and closed arm and time spent in open arm are significantly shown in mice behavior activity,similarly histopathological study also showed that pretreatment of antioxidant vitamin.E prevent necrosis and cellular membrane damaged and massive diffused cells from that of plant extract admistrated group. similar result with ameliorating effect of vitaminE in brain was reported(Goudarzvand et al, 2010). The biochemical parameters of mice brain compared to plant extract treated group show highly protected in vitaminE pretreated group as shown. Similar results was reported by (Halliwell and Gutteridge 1985: Fariss and Zhang 2003: Gupta et al, 2011) with vitamin E attenuates the effects of lipid peroxidation by trapping these free radicals. (Lan and Jiang, 1997) reported reduced in lipid peroxidation with vitaminE pretreatment. some reports have shown the efficacy of vitamin E in exerting neuroprotective effect by decreasing the rate of lipid peroxidation (Inci et al, 1998: Aiguo et al, 2010).In the present study GOT and GPT activity decline in pretreated group of vitaminE with plant extract when compared to plant extract treated group alone. Our result are in consonance to the study of (Osfor et al,2010) in which vitaminE diminished GOT and GPT level in Pb and Cu intoxicated male rats in the tissue of liver and kidney. Similarly, (Sajitha et al, 2010) reported declined in histopathological and biochemical alterations induced by Pb intoxication in female Sprague-Dawley albino rats after administration of vitamin E. According to (Leonarduzzi *et al*., 2010) alphatocopherol is the most potent antioxidant that acts upon cell membranes and has the ability to neutralize compounds which may potentially disrupt membrane stability. The protective effect of Vit E against the neurotoxic effect of *Plumbago zeylanica* may be attributed to the ability of Vit E as the first line of defense against peroxidation of polyunsaturated fatty acid contained in cellular and subcellular membrane phospholipids. VitaminE act as antioxidant breaking free

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radical chain reaction as a result of their ability to transfer a phenolic hydrogen to peroxyl free radical of peroxidized polyunsaturated fatty acid (Murry *et al*., 2000).

The result of present study shows effect in behaviour and histological profile and alteration in biochemical parameters after treatment with *Plumbago zeylanica*, the reason may be that plant extract admistration causes oxidative stress in mice brain with altered biochemical activities thereby causing adverse toxicity in the central nervous system, antioxidant Vitamin E induced protective against *Plumbago zeylanica* neurotoxicity by restoring biochemical activity with significant decline in LPO, GOT and GPT activity and ameliorated manifestation in histopathological study.From the present study it can be concluded that antioxidant vit.E have profound ameliorating effect against neurotoxic effect. However, neuroprotective effect of Vitamin E should be relook in more parameters and the selected dose in this experiment may not apply for human application in real human life as the dose range may varies from person to person.

2.12 *Conflict of interest*

The authors declear that there is no conflict of interest**.**

3. ACKNOWLEDGEMENT

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