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RESEARCH ARTICLE

EVALUATION OF BEHAVIOUR AND BIOCHEMICAL PARAMETERS IN METHANOL ROOT EXTRACT OF PLUMBAGO ZEYLANICA L. IN EXPERIMENTAL MICE

[1]Paojatong Singson and [2]Meenakshi Bawari

[1]Department of Life Science, Assam University, Silchar. Neurobiology laboratory
[2]Department of Life Science, Assam University, Silchar.

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ABSTRACT

In the present study we evaluated the effect of methanol root extract of *Plumbago zeylanica* L. on central nervous system. The methanol root extract of *Plumbago zeylanica* was investigated in a battery of behavior models (hole board, locomotor, and forced swim) in mice. Roots of *Plumbago zeylanica* L. have several therapeutic applications in folk medicine some authors also reported the poisonous and toxic properties of the plant roots. In the present study methanol root extract produced significant effect on the nervous system at the doses of 160 mg/kg (i.p route) compared to reference drug diazepam (DZP) and fluoxetine. The brain catalase activities was significantly decreased ($p<0.05$) and Lactate dehydrogenase enzyme increased significantltly ($p<0.05$) when compared the treated group with control. The catalase and LDH activities of cerebral cortex region showed highly significant (**$p< 0.01$) when compared with mid brain region in treated group. In conclusion, results suggest that the methanol root extract of *Plumbago zeylanica* L. possess potent effect on nervous system and disturbances in enzyme activities in mice brain.

INTRODUCTION

Herbal products are extensively used globally for the treatment of many diseases with the hope that they have lesser side effects than with synthetic drugs. The plant *Plumbago zeylanica* L. is commonly known as white leadwort, it is distributed in several parts of India, cultivation of this plant is widely practiced due to its more therapeutic use (Chetty, 2006). Several pharmacological study was carried out by different workers and *P.zeylanica* was reported to possess hepatoprotective, antioxidant, hypolipidaemic and anti-atherosclerotic properties,(Bickford *et al*,2000; Bopiah *et al*,2001; Itoh *et al*,1990; Jarvik *et al*, 1995). All parts of the plant are used for pharmacological activity the aerial parts or pulped roots are used as abortifacient, laxative, expectorant, appetizer, while powered bark of root or leaves are employed for syphilis, gonorrhea, tuberculosis, rheumatic pain, swellings and wound healing (Thakur *et al*,1989; Bhattacharjee, 1998). The root has been reported to have numerous therapeutic uses (Chetty, *et al*, 2007), viz, antifertility, antidermal, diuretic, antimicrobial, antiulcer and antidiarrhoeal activities. it is also reported to possess anticancer (Nguyen *et al*,2004; Xu & Lu 2010 and anti-tumor (Yang *et al*,2010). Recent studies have shown that herbal drugs exert good sedative and hypnotic effect on the central nervous system (Huang *et al*, 2007; Herrera-Ruiz*et al*, 2007; Pérez-Ortega *et al*, 2008). The roots of this plant has been reported to be a powerful poison when given orally or applied to ostium uteri, causes abortion (Azad Choudhary *et al*,2005).Therefore the present study is an attempt to evaluate the toxic effects of plumbago zeylanica root extract on chronic exposure to the treated mice.

Experimental procedure

P.zeylenica plant roots was collected from Nagaland. The plant was authenticated from Botanical survey of India (BSI) Shillong. The roots were cut into pieces, shade dried and then grinded to powdered and macerated with 80% methanol for 72 hrs and subsequently filtered using watmann filter paper. Healthy adult male albino mice weighing 25±3 gm b.w were collected from Pastuer institute Shillong for the experiment study. The animals were fed on standard balanced diet. Experimental animals were handled according to the guide lines of Assam University ethical committee. LD50 was detrmined by intraperitoneal (i.p) route using the method of Lorke (1983). and 750 mg/kg (i.p) is considered as lethal dose in methanol root extract of *P.zeylenica* in the present experimental study. All the animals were randomly divided

Corresponding author:* **Paojatong Singson
Department of Life Science, Assam University, Silchar. Neurobiology laboratory

into three groups, each containing five mice. The groups of mice were treated as follows: (i) control (distilled water); (ii) MEPZ (160mg/kg); (iii) diazepam (1mg and 2mg/kg) / fluoxetine (10mg/kg). Diazepam and fluoxetine were dissolved in distilled water immediately prior to use. All administrations were performed intraperitonally to the respective groups for a period of fifteen days. The experiments were performed one hour after the administration of last dose at the end of 15 days, behavior study was done on three parameter i.e hole board, forced swim and locomotory test. Hole board test was done by method of Clark *et al*, (1971) by counting the number of head dips in the explored holes during 5min observation and Diazepam (1mg/kg) was used as standard drug, forced swim test was followed by porsolt *et al*, (1978),fluoxetine was used as standard drug in which total period of immobility or cessation of swimming with head just floating above water level was determined during 5 min observation, similarly, Locomotory study was carried according to Quafa and Nour,(2008). In which the number of crossed squares was recorded for each mouse for 10 min (5+5 min).

Diazepam (2mg/kg i.p.) was used as the positive control drug. Just after the behavioral study the mice in the experimental and control groups were sacrificed quickly by cervical dislocation. The brains were removed and cerebral cortex and mid brain were then separated immediately. They were washed quickly with saline, blotted between two damp filter papers and then weighted using electronic balance. LDH activity was assayed in tissue homogenate by method of Cheng T. C (1989).

Enzyme activity is expressed as units/min/mg protein at 25ºC.and Catalase activity was assayed colorimetrically as described by Sinha,(1972) using dichromate acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1:3 ratio). The intensity was measured at 620 nm and the amount of hydrogen peroxide hydrolyzed was calculated for the catalase activity. Enzyme activity is expressed as µmoles/min/mg protein. Protein was estimated by Lowry *et al*.(1951) for calculating specific activity of the brain enzymes. Statistical values were evaluated by one way ANOVA followed by LSD multiple comparisons test (*P<0.05 & **P≤0.01 vs Control).

RESULT AND DISCUSSION

In the present study Acute toxicity of methanol root extract of *P.zeylenica* in mice was 750 mg/kg body weight, intraperitoneally, Pharmacological tests was then performed at nontoxic doses (i.e 160 mg/kg, i.p.), for the methanolic root extract. In the present study, mice treated with methanol root extract of *P.zeylanica* at 160 mg/kg i.p, produced a significant (*p< 0.05) decrease in the number of head dips when compared the treaeted group with control as shown in fig.1. Mice that received diazepam 1 mg/kg i.p significantly increased the number of head dips when compared with the control (** p< 0.01).similarly a decrease in number of head dips with sedative behaviour was reported by File and Pellow, (1985) and measure of CNS depressant activity by Adzu *et al*, (2002); Viswanatha *et al*, (2006).

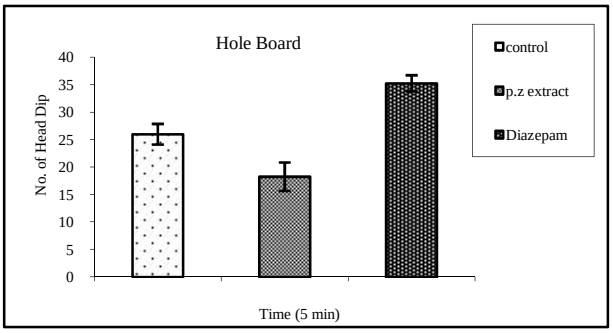

Figure 1 Effects of *P.zeylanica* & Diazepam (1mg/kg) on the number of head dips in the hole-board apparatus. Each column represent mean±SEM (n=5). Comparisons were made by using one way ANOVA followed by LSD Multiple Comparison test (P<0.05 vs.control).

Mice treated with methanol root extract of *P.zeylenica* at 160mg/kg, i.p showed significant (*p< 0.05) decreased in locomotory activity when compared with control as shown in fig.2 and diazepam 2mg/kg i.p also decreased the locomotor activity to a great extent significantly (** p< 0.01) when compared with the control. Several author also reported decreased locomotor activity related to sedation Ayoka *et al*, (2006), Ozturk *et al*, (1996) and locomotor activity as a measure of CNS excitability by Ashraful *et al*,2008. Similarly Gottesmann (2002) also reported sedative activity of methanolic and aqueous extract of Lavandula officinalis.

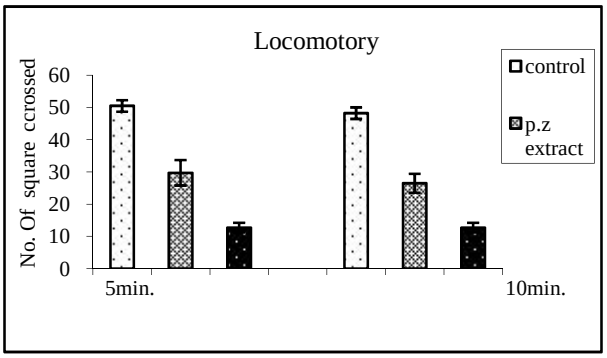

Figure 2 Effects of *Plumbago zeylenica* & Diazepam (2mg/kg) on locomotor activity. Each column represent mean±SEM (n=5).Comparisons were made by using one way ANOVA followed by LSD Multiple Comparison test (P<0.05 vs.control)

In force swim test the immobility time was increased significantly in methanol root extract of *plumbago zeylanica* treated mice when compared with the control (*p< 0.05) and fluoxetine treated group also showed significantly decreased in immobility time when compared with the control group(**p< 0.01) as shown in fig.3. similarly result was reported by Rebai & jebli,2008 with decreasing immobility due to Aluminum chloride toxicity. Increase oxidative stress in the cell has often been shown to cause alterations in antioxidant enzymes (Skaper, 1997). Catalase is a ubiquitous antioxidant enzyme found in all known organisms and it provides protection against oxidative stress Berntssen *et al* (2003). It catalyzes the break down of H202 to H20 and molecular

oxygen Hugo (1994). Oxidative modifications could lead to neuropathology, neuronal dysfunction and cell death Siedlak (2009).

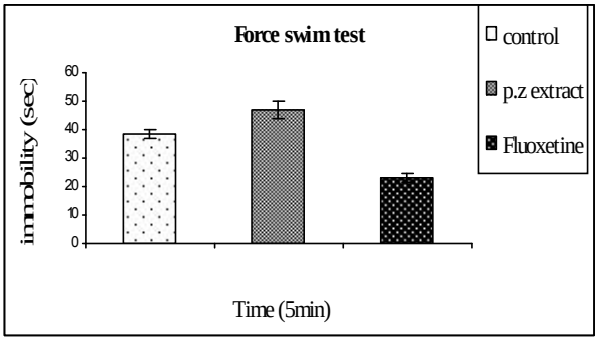

Figure 3 Effects of *Plumbago zeylenica* & Fluoxetine (10mg/kg) on immobility time in forced swimming test. Each column represent mean±SEM (n=5). Comparisons were made by using one way ANOVA followed by LSD Multiple Comparison test (P<0.05 vs.control).

Therefore, an excess production of Reactive oxygen species exerts toxic effects in the cells. The antioxidant enzymes like catalase have an important function in mitigating the ROS. In the present study catalase activity was found to decrease in treated mice when compared to control in both Cerebral cortex and midbrain. Here cerebral cortex showed more significant change (P<0.001) than midbrain (p< 0.05) as shown in Fig 4. Similar result was reported with decreased catalase activities with fluoride accumulation in brain of albino mice Vani *et al*., (2000) and reduced catalase activity with Taurine in rat brain Ward *et al*., (2001) also Adenuga *et al*, (2009). reported reduced brain catalase activity related to neurodegenerative diseases.

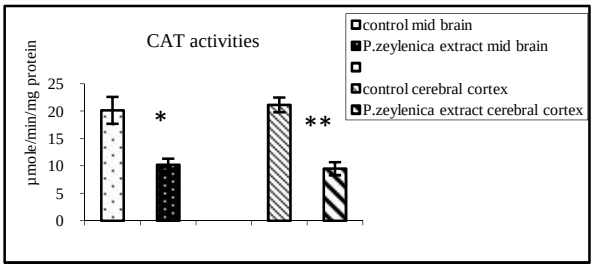

Figure 4 Effect on catalase activity in cerebral cortex and mid brain regions following MEPZ administration. Each column represent mean±SEM (n=5). Comparisons were made by using one way ANOVA followed by LSD Multiple Comparison test (* P<0.05; ** p< 0.01, vs.control).

The extracellular appearance of LDH is an important indicator showing cell damage or cell death Nemensanszky and Lott (1987).In the present study LDH was highly elevated in methanol root extract treated group when compared with the control as shown in fig,5. alteration in LDH level was significant in cerebral cortex region (**p< 0.01) than in mid brain region(*p< 0.05) of the treated group, the changes in LDH level indicated metabolic changed in stressed mice which may be due to treatment of p.zeylanica extract . Similar result of increased in LDH activity was obtained in albino rats treated with sodium selenete Vasantha Sena (2002) . in albino mice treated with sodium fluoride Manna *et al*,(2004). John Sushma *et al*,(2007) also reported similar increase in LDH activity in albino mice treated with aluminium acetate, also elevation in LDH with cellular metabolic activities was reported by Abston, and Yarbrough, (1976).

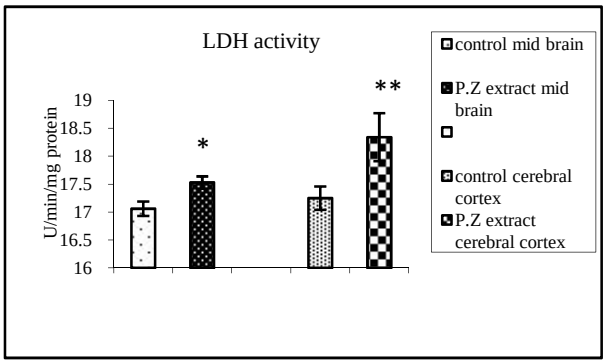

Figure 5 Effect on LDH activity in cerebral cortex and mid brain regions following MEPZ administration. Each column represent mean±SEM (n=5). Comparisons were made by using one way ANOVA followed by LSD Multiple Comparison test (* P<0.05; ** p< 0.01, vs control)

CONCLUSION

From the present study the overall results revealed that the methanol root extract of *P.zeylanica*. possess biologically active principles that have profound effect on nervous system. Similarly investigation on biochemical parameter viz, catalase and LDH showed that plant extract is active in producing sufficient toxic effects on the treated mice at 160 mg/kg b.w. Thus, from the present study it can be concluded that prolonged use of *P.zeylanica* plant extract may cause neurotoxicity, further studies are required in selecting dose for prolonged theurapeutic usage.

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Conflict of interest

The author declear that there is no conflict of interest.

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