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Evaluation of The Harmful Effect of a Medicinal Plant Extract On Micebrain

KEYWORDS: medicinal plant, behavior, enzymes, Alstonia scholaris, harmful.

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ABSTRACT *The present study aims to assess the harmful effect of a selected medicinal plant extract on albino mice and investigate its impact on its behavior and brain enzymes. Here force swim test showed significantly dose dependent increase immobility. But in case of locomotor activity there found significant decrease in experimental group. Besides significant elevation of Lactate dehydrogenase activity and reduction of Catalase activity of brain was found, which showed the negative effect of the extract. Therefore, it can be assumed that the Alstonia scholaris extract has some harmful effect on mice brain.*

Introduction

The plant Alstonia scholaris is a medicinal plant used against different diseases. Its extract can cure asthma, hypertension, lung cancer, pneumonia, fever (Holdsworth, 1986). It is also reported as a stimulant, carminative, stomachic, expectorant and febrifuge in earlier study (Nadkarni, 1976). On the other hand Baliga et al., (2004) found toxic effect of A.scholaris extract. It has been seen that toxins of various plants are involved as final common mediators of neuronal death associated with various types of neurotoxic insult (Dawson, 1995). Lathyrism, one of the oldest neurotoxic disease known to man result from excessive consumption of the Chickling pea, Lathyrus sativus and certain related species (Spancer,1986). Behavioural outcome indicates the status of internal physiological environment of an animal brain. Enzyme is another key factor between brain and behavior. Here both behavioral and biochemical tests has been performed to sketch a brief idea about the possible toxic effect of the plant extract.

MATERIALS & METHOds

The fresh stem bark of Alstonia scholaris was collected from the nearest forest of Dargakona area belongs to Cachar district of Assam, India and identified by "Assam University Herbarium collection centre". Than the extract was prepared by dipping the grind material in distilled water (Akindele & Adeyemi, 2010). The Swiss albino mice (25-30g) were collected from Pasteur institute, Shillong. The handling and experiments of animals were according to the rules and guidelines of "Assam University Animal Ethics Committee". After acclimatization mice were divided into three groups (6 in each) and treated for seven days. The first group (Control) was getting intra peritoneal injection of distilled water. Similarly second group (low) got 100 mg/kg (body weight) and third group (high) got 200 mg/kg (bw) A. scholaris aqueous extract. (Baliga et al, 2004). Besides two another group of animal was taken treated by Diazepam (1mg/kg, ip.) and Fluoxetine (20 mg/kg, ip.) for compared as standard drug (Wattanathorn et al., 2006). Than 2 behavioral experiments was performed ie. Force swim test and Locomotor activity. Force swim test was performed by Porsolt et al., 1977. The antidepressant activity in immobility (second) of swimming was assessed for 5 minute. Fluoxetine was used as standard drug. In locomotor activity test Rebai & Djebli, 2008 procedure was used. The number of crossed squares was recorded for each mouse per time of 5 min for 20 min investigation. Diazepam was used as standard drug. After that the mice were sacrificed and cerebral cortex and midbrain were separated and washed with saline for biochemical assay. Than the tissue were homogenized with 0.05 M phosphate buffer for performing catalase assay (Aebi,1974). Similarly another portion of both the tissue were homogenized in 0.32M sucrose for performing lactate dehydrogenase assay (Wroblewski & Ladue, 1955). Protein was also measured by Lowry et al., 1951 for calculating specific activity of the brain enzymes. The values were evaluated by one way ANOVA along with Students-Newman Keuls, Turkeys multiple comparison test (*P<0.05 & **P≤0.001 vs Control).

Results & Discussion

Among all animal models, the forced swim test (FST) remains one of the most used tools for screening antidepressants (Demouliere et al., 2005). Here the plant extracts treated groups showed significant (P≤0.001) increase in immobility time of the test. On the other hand Fluoxetine treated group developed a significant (P<0.05) decreased duration which was compared as standard drug (Fig 1). Rebai & Djebli, 2008 found similar result of decreasing immobility due to Aluminum chloride toxicity. It reflects the depressant activity of the extract.

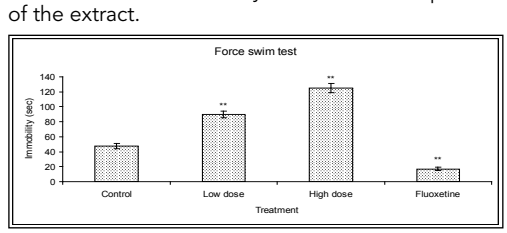

Fig 1: Histogram (Mean±SE, N=6) of immobility time in Forced swimming test.

Locomotor activity is considered as an index of alertness and a decrease in it, is indicative of sedative activity (Ozturk, et al., 1996). In this study locomotor activity got significantly (P≤0.001) decreased in dose dependent manner than control, resulting sedation. There found significantly decreased score for Diazepam induced group also where P value ≤0.001 as it is a sedative drug.(Fig 2)

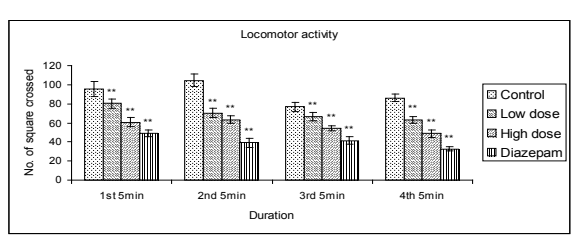

Fig 2: Histogram (Mean±SE, N=6) of locomotor activity.

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Oxidative modifications have been proposed as one biochemical change that could lead to the neuropathology, neuronal dysfunction and death (Siedlak, 2009). Under normal condition, mitochondria consumed a little oxygen and convert to free radicals like hydroxyl radical and other reactive oxygen species (Vora, 2009). An excess production of ROS is harmful to a cell which is likely to exert toxic effects in the cells. The antioxidant enzymes like catalase have an important function in mitigating the ROS. Increase oxidative stress in the cell has often been shown to cause alterations in antioxidant enzymes (Skaper, 1997). Here catalase activity was found decreased in dose dependent manner compared to control treatment in both Cerebral cortex and midbrain. Here cerebral cortex showed significant change in both the doses ($P<0.001$) and midbrain also showed significant change in both the doses (low $P<0.05$ and high $P<0.001$) (Fig 3). Similar result was found by Vani et al., (2000) where catalase activity was decreased due to fluoride accumulation in brain of albino mice which probably make the tissue more susceptible to biochemical injury. Ward et al., (2001) also reported that brain catalase activity reduced due to chronic administration of Taurine in rat brain. Reports have shown an association between reduced catalase activity and neurodegenerative diseases (Adenuga et al., 2009).

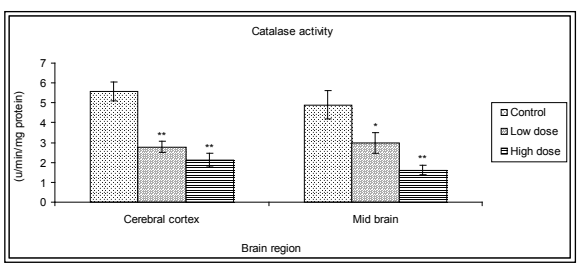

Fig 3: Histogram (Mean±SE, N=6) of Catalase activity.

LDH brings about the reversible conversion of pyruvate to lactate. The levels of this enzyme are indicative of the oxidoreductive state of the tissue (Maker et al., 1976). There found dose dependent increased score in LDH activity in this study. Both the region showed significant increase in both the doses ($p<0.001$) (Fig 4). Hanan et al., (2004) also showed a marked disturbance in the oxidative status of rat brain exposed to Rotenone. They observed a 1.5 fold elevation in the level of LDH in comparison to control. Dua et al., (2010) also reported that the acute administration of Aluminium phosphate increased the LDH activity of rat brain. Under anaerobic conditions, the reoxidation of NADH to NAD+ is achieved by the conversion of pyruvate to lactate by LDH without any concomitant ATP production. This would stimulate LDH, resulting in an accumulation of lactate in the brain of animals, which may cause neurologic deficits.

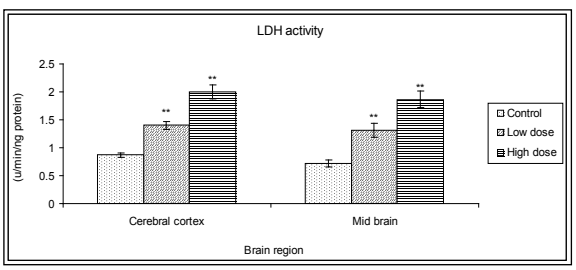

Fig 4: Histogram (Mean±SE, N=6) of LDH activity.

Conclusion

The extract of A.scholaris showed CNS depressant activity & sedative behavior on mice. It creates oxidative stress in mice brain by decreasing catalase and increasing LDH. Besides elevated LDH is a cell death marker (Lott, 1987). Above all it can be summarized that A. scholaris in higher concentration created a marked toxicity to the brain of mice. As brain is the preliminary and main responsive organ of nervous system so it is considered for critical toxic contamination. As because it is a medicinal plant, it become important to investigate the active constituent and mode of toxicity further. The users of this natural resource have to be careful while selecting the dose and mode of administration by detail pharmacological screening in demand of avoids its negative effects.

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