



Research Paper

STUDY ON NEUROTOXICOLOGICAL EFFECT OF *Biden pilosa* L. LEAF EXTRACT ON MALE ALBINO MICE

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Abstract

Biden pilosa L. (Asteraceae) is a world-wide medicinal plant used in several medication by mankind, the present study is designed to investigate probable neurotoxic properties related with long term use of this medicinal plant, neurobehavioral and biochemical parameters CAT, LDH and GDH activity is evaluated to determine neurotoxic effects. Control mice were intraperitoneally treated with vehicle (distilled water) and positive control mice with diazepam (1mg/kg and 2mg/kg i.p). Mice treated with the extract (130 mg/kg intraperitoneally) did not showed much changed in both locomotor and hole board activity from that of the control animals. The effect of intraperitoneal administration of the plant extract on the activities of catalase (CAT) and lactate dehydrogenase (LDH) in the brain tissues of the experimental animals were analyzed and compared with that of the control. The extract showed a significant decrease in CAT activity whereas there was a significant increase in the LDH activity.

Key words: Medicinal plant, *Biden pilosa*, Neurotoxic, Catalase, Lactate dehydrogenase, Glutamate dehydrogenase.

INTRODUCTION

The use of herbal medicines as alternative treatments has been increasing worldwide and gaining popularity in developing countries[1]. Although medicinal plants may have biological activities that are beneficial to humans, the potential toxicity of these bioactive substances has not been well established [1]. Thus, the safety and efficacy of these plants must be studied thoroughly to maximise their benefits for mankind. The increase in number of users as oppose to the scarcity of scientific evidences on the safety of the medicinal plants have raised concerns regarding toxicity and detrimental effects of these remedies [2]. and Therapeutic application of herbal preparations are prevalent in many countries of the world [3,4]. Recent Scientific studies, although not extensive, have demonstrated that *B.pilosa* extracts and or compounds have anti-tumor [5,6,7,8,9], anti-diabetic and anti-hyperglycemic [10,6], antioxidant [6,11], antimalarial[7,12], anti-bacterial[12,6,13], anti-fungal[13,14,], anti-hypertensive, vasodilatory [15,16], and anti-ulcerative [15] activities. Apart from the pharmacological study as seen in literature by different workers the effects of medicinal plants on nervous system function have received considerably less attention in its association to neurotoxicity, and no toxicological study on central nervous system was done by earlier author in *Biden pilosa* L.. Therefore, the aim of present study is to investigate neurotoxic effect of *Bidens pilosa* L. and to evaluate its effects on mid brain and cerebral cortex of treated mice.

MATERIAL AND METHODS

For the present investigation fresh leaves of *Biden pilosa* was collected from Nagaland, in Ahthibung local forest and identification and authentication was done in BSI shillong. The fresh leaves of the plant was collected and shade dried for 30-40 days and grinded them to powdered form using electronic grinder. Male albino mice 27 ± 3 g were selected for the present study. Animals were housed five per cage with free access for food and water *ad libitum*. The animals were maintained in the animal house with day and night cycle of 12 hours. The food provided to animals was standard laboratory feed. Experimental animals were handled according to the guide-lines of Assam University Ethical Committee and supervision of experiments on animal. All the hygienic practice was followed during the maintenance of animals. Lethal doses (LD50) of *Biden pilosa* were determined [17]. For the present study animals were administered 130 mg/kg body weight intra-peritoneal route for 15 days. All the animals were randomly divided into three groups each containing five mice. The groups of mice were treated as follows: (i) control (distilled water); (ii) AEBp (130 mg/kg i.p.); (iii) diazepam (1mg/kg i.p). Diazepam was dissolved in distilled water immediately prior to use. The experiments were performed one hour after the administration of last dose at the end of 15 days. Behaviour study, Hole board test was done [18] by counting the number of head dips in the explored holes during 5min observation and Diazepam (1mg/kg i.p) was used as standard drug, and Locomotory study [19] the number of crossed squares was recorded for each mouse for 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. Lactate dehydrogenase content was estimated [20] and enzyme activity expressed as units/min/mg protein at 25°C. Catalase content was estimated [21] and catalase activity was expressed as μ moles of H₂O₂ consumed/min/mg protein. [22] Glutamate dehydrogenase activity was estimated and enzyme activity was expressed as unit/min/mg protein. Protein was also measured [23] for calculating specific activity of the brain enzymes. The values were evaluated by one way ANOVA along with Least significant difference (LSD), with multiple comparison test (* $P < 0.05$ & ** $P \leq 0.01$ vs Control). All the values were subjected to statistical treatment.

RESULT AND DISCUSSIONS

In the present study acute toxicity of aqueous extract of *Biden pilosa* in mice was 650 mg/kg body weight intra-peritoneal, Pharmacological tests was then performed at non-toxic doses (i.e 130 mg/kg, i.p.), for the AEBp. In the present study, *Biden pilosa* treated group produced a significant ($p < 0.05$) decrease in the number of head dips when compared 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 ($p < 0.05$). Similarly, decrease in number of head dips with sedative behaviour was reported [24] and measure of CNS depressant activity [25,26]. Locomotor activity is considered as an index of alertness and a decrease in it is indication of sedative activity [27] and it is a measure of CNS excitability [28]. In the present study mice treated with aqueous leaves extract of *Biden pilosa* at 130 mg/kg, i.p showed decreased in locomotory activity when compared with control ($p < 0.05$) as shown in fig.2 and diazepam 2mg/kg i.p also decreased the locomotor activity to a great extent significantly ($p < 0.05$) when compared with the control. Several author also reported decreased locomotor activity related to sedation [29,27]. Similarly, sedative activity of methanolic and aqueous extract of *Lavandula officinalis* was also reported [30].

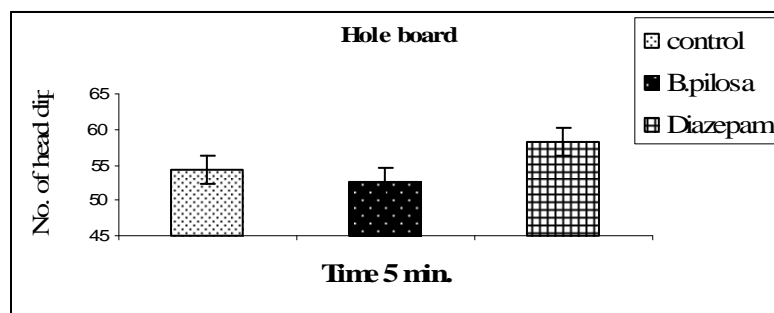


Figure 1: Effect of *Biden pilosa* & Diazepam (1mg/kg) on the number of head dips in the hole-board apparatus. Each column represent mean \pm SEM (n=5). Comparisons were made by using one way ANOVA followed by LSD Multiple Comparison test ($P<0.05$ vs.control).

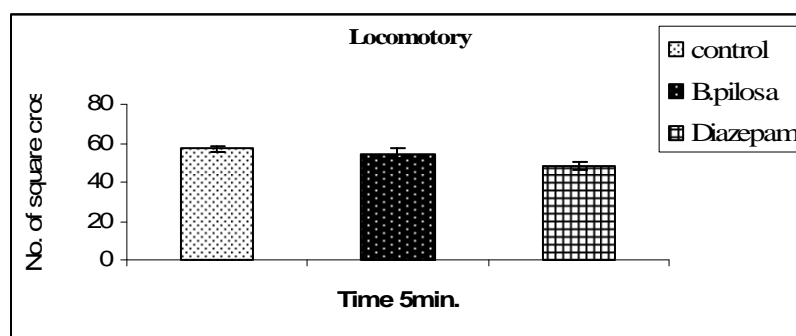


Figure 2: Effect of *Biden pilosa* & Diazepam (2mg/kg) on locomotor activity. Each column represent mean \pm SEM (n=5). Comparisons were made by using one way ANOVA followed by LSD Multiple Comparison test ($P<0.05$ vs.control).

Oxidative modifications have been proposed as biochemical change that could lead to the neuropathology, neuronal dysfunction and death [31]. Under normal condition, mitochondria consumed a little oxygen and convert to free radicals like hydroxyl radical and other reactive oxygen species [32]. 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 anti-oxidant enzymes [33]. In the present study catalase activity was significantly decreased in the treated group compared to control in both Cerebral cortex and midbrain. Cerebral cortex showed highly significant change in the treated group ($**P<0.01$) and change in the mid brain was also significant ($*P<0.05$) (Fig.3). Similar result was found [34] where catalase activity was decreased due to fluoride accumulation in brain of albino mice which probably make the tissue more susceptible to biochemical injury. Similarly [35] 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 [36].

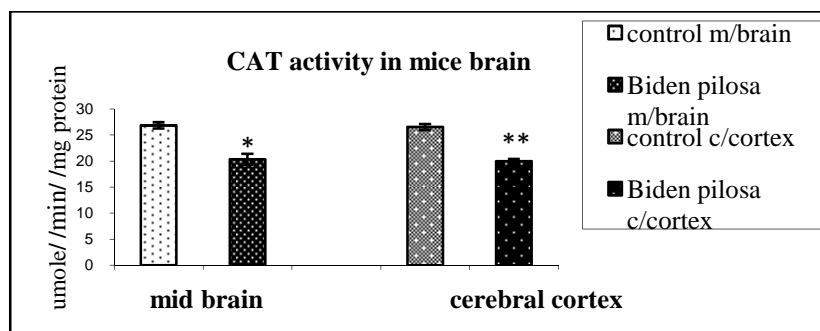


Fig.3. Effect of *Biden pilosa* aqueous extract (130 mg/kg i.p) on Catalase activity in mice brain, values are mean \pm S.D. No. of animals in each group (n=5). comparison were made by using one way ANOVA followed by LSD multiple comparison test. *p<.05, **p<.01 value are considered statistically significant with control.

The extra-cellular appearance of LDH is an important indicator showing cell damage or cell death [37]. Increased activity of LDH is a characteristic feature of a shift from aerobic to anaerobic metabolism leading to an elevated rate of pyruvate conversion into lactate, resulting in lactic acidosis [38]. The LDH activity increases during conditions favoring anaerobic respiration to meet energy demands, when aerobic respiration is lowered [39]. In the present study LDH was highly elevated in *Biden pilosa* treated group when compared with the control as shown in fig.4, 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 elevation in LDH level may be due to treatment of *Biden pilosa* extract which may be due metabolic changed in stressed mice. Increased LDH activity was supported by several authors. Albino rat treated with sodium selenite [40], with arsenite treatment in fresh water fishes [41], in albino mice with sodium fluoride [42] and in kidney of young pigs [43]. Similar increase in LDH activity was reported in albino mice treated with aluminium acetate [44].

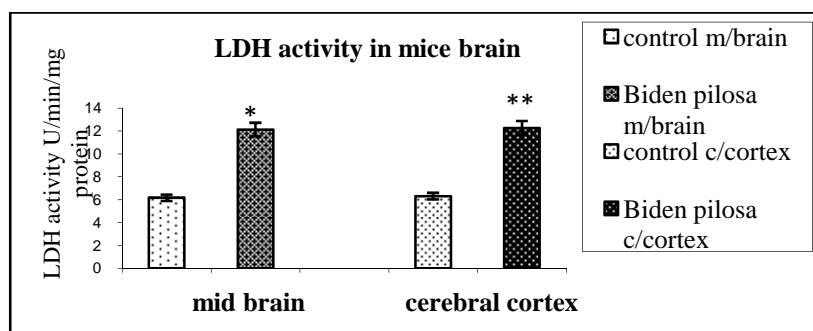


Fig.4. Effect of *Biden pilosa* aqueous extract (130 mg/kg i.p) on LDH activity in mice brain, values are mean \pm S.D. No. of animals in each group (n=5). comparison were made by using one way ANOVA followed by LSD multiple comparison test. *p<.05, **p<.01 value are considered statistically significant with control.

GDH is a mitochondrial enzyme, catalyzes the oxidative deamination of glutamate generating α -ketoglutarate, an important intermediate of the TCA cycle. In the present study increase in GDH activity was significantly in cerebral cortex (**p< .01) than in the mid brain region (*p< .05) after fifteen days of treatment with *Biden pilosa* extract compared to control. Similarly progressive increase was reported in the activities of GDH in all the organs of the fish exposed to cypermethrin, [45,46].

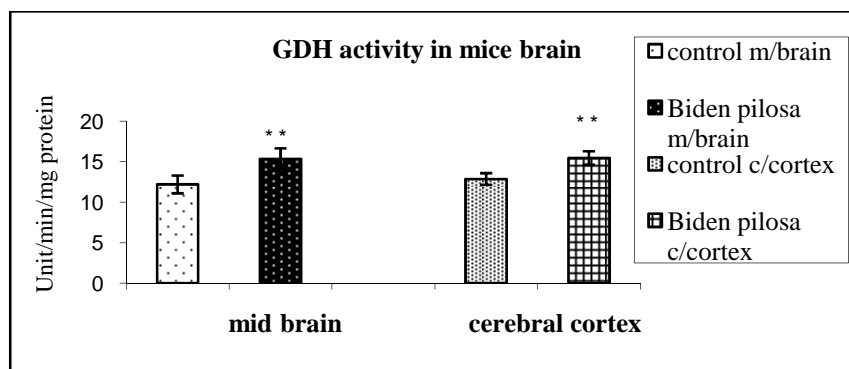


Fig.5. Effect of *Biden pilosa* aqueous extract(130 mg/kg i.p) on GDH activity in mice brain, values are mean \pm S.D. No. of animals in each group (n=5).comparison were made by using one way ANOVA followed by LSD multiple comparison test. *p<.05, **p<.01 value are considered statistically significant with control.

The increase in GDH activity indicates either increased mitochondrial permeability or the lysosomal damage or the induced synthesis of enzymes [47].The increased levels of GDH activity in *Biden pilosa* treated animals may be due to increased mitochondrial permeability in the present investigation. Increased, GDH activities are reported by several authors in mice treated with sublethal dose of aluminium acetate [44] cypermethrin toxicity in fish [48], in albino rats exposed to hexachlorophene [49], [50] reported increased GDH activity in liver tissues of rat after acute poisoning with sodium fluoride.

The results of the present study neurobehavioural study do not showed much changed in hole board and locomotory activity when compared to control but significant alterations in biochemical enzymes activities in mice brain was noted which may be due to intoxication of *Biden pilosa* extract in albino mice. Further, it can be stated that long term exposure to sublethal doses of *Biden pilosa* can result in cell metabolism toxicosis, leading to physiological impairment. Therefore, further study is necessary for appropriate dose selection for further use of this medicinal plant.

CONFLICT OF INTEREST

The author declare that there is no conflict of interest

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