CHARACTERIZATION OF THE TOXIC EFFECTS INDUCED BY DATURA STRAMONIUM L. LEAVES ON MICE: A BEHAVIORAL, BIOCHEMICAL AND ULTRASTRUCTURAL APPROACH

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Received:14 May 2012, Revised and Accepted:21 June 2012

ABSTRACT

This study was designed to document toxic properties of aqueous extract of Datura stramonium L. leaves (AEDSL) by investigating the neurobehavioral, biochemical and ultrastructural alterations using mice model. The extract was studied in several paradigms which included locomotor activity, forced swimming test and hole-board test. Control mice were intraperitoneally treated with vehicle (distilled water) and positive control mice with diazepam (1mg/kg and 2mg/kg i.p) and fluoxetine (10mg/kg i.p). Mice treated with the extract (20mg/kg & 40mg/kg intraperitoneally) showed decrease in locomotor activity. Forced swimming test revealed that the extract was able to promote significant increase in the immobility time. In the hole-board assay, it caused decrease in the number of head dips 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. The cerebral cortex of the mice treated with the extract was studied under transmission electron microscopy. The photomicrograph of the sections demonstrated various prominent ultrastructural changes. AEDSL possessed remarkable central nervous system depressant properties, altered biochemical parameters and have potential to cause damage to the ultrastructure of the brain cells. However further study is needed for pharmacological and toxicological characterization.

Keywords: Datura stramonium, aqueous extract, TEM, neurobehavioral, enzymes

INTRODUCTION

Datura stramonium, a member of the family Solanaceae commonly known as Jimson weed has been documented as a annual herb with medicinal properties, but poisoning readily occurs because of misuse. The Datura genus is known for anti-asthmatic, sedative and anticholinergic properties. Mixture of the leaves and seeds taken orally as a decoction or smoke is used as a cure for the asthma. Aqueous extract of the seeds are reported to be used in the treatment of gastric pains and indigestion. It has been reported that the whole plant extract of D.stramonium is toxic and thus is used for their insecticidal and antifeedant properties against Dysdercus cingulatus Fabricius (Hemiptera:Pyrrhocoridae), Spodoptera litura Fabricius (Lepidoptera:Noctuidae), and Pericallia ricini Fabricius (Lepidoptera:Noctuidae). When D.stramonium extracts were used for their acaricidal activity against T.urticae under laboratory conditions, the compound was toxic to all stages of the spider mite. Recently, it has been used as a narcotic and local anesthetic drug in many societies and in some nations young people use its leaves by smoking for hallucination purpose. Previous studies have reported that this plant contains a variety of alkaloids including atropine, hyoscyamine and scopoaline that can cause anticholinergic poisoning if taken in large concentrations. However it is also these anticholinergic alkaloids that contribute to the anti-asthmatic properties and it is therefore classified as a plant with anticholinergic properties. The brain is metabolically one of the most active organ in the body and much more susceptible to free radical attack and oxidative stress. Lactate dehydrogenase (LDH) is a principle biomarker of toxic stress and is also found to be involved in energy production. Despite the various pharmacological activities of this plant that have been reported, no study combining the neurobehavioral, biochemical and ultrastructural changes from AEDSL have so far been undertaken. There is little information in the literature regarding the proper usage such as dosage, frequency and usage period and sensitivity of the user. Moreover, it is important that medicinal plants which have folklore reputation for medicinal effects should be investigated in order to establish their safety and efficacy.

MATERIAL AND METHODS

Plants materials

Fresh leaves of the D.stramonium were collected, washed and dried under room temperature for about 30 days.

Preparation of the extract

The air-dried leaves were minced into small pieces and macerated in distilled water (70 g in 700ml) and the extract was decanted after 24 hour. The filtrate was evaporated to dryness in the oven at 40°C. The dried extract was weighed and dissolved in distilled water to give the required concentration before administration to the experimental animals.

Animals

Adult male albino mice weighing between 26g and 32g, used for the study were obtained from the Pasture Institute, Shillong. The animals were housed in cages under standard environmental conditions and had free access to food and water ad libitum. The experiments were performed in accordance with the guidelines in the care and use of laboratory animals and were approved by the Ethical Committee of the Assam university, Silchar.

Experimental protocol

All the animals were randomly divided into four groups, each containing six mice. The groups of mice were treated as follows: (i) control (distilled water); (ii)AEDSL (20mg/kg); (iii)AEDSL (40mg/kg); (iv) diazepam (1mg and 2mg/kg) / fluoxetine (10mg/kg). Diazepam and fluoxetine were dissolved in distilled water immediately prior to use. All administrations were performed intraperitoneally to the respective groups up to a volume of 5 ml/kg body weight for a period of seven days. The experiments were performed one hour after the administration of last dose.

Locomotor activity

All mice were tested in acrylic cages (45×25 cm) divided into 16 equal squares. 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.

Hole-board test

The hole-board apparatus consisted of a wooden box (60 × 60 × 35 cm) with four equidistant holes 4 cm in diameter in the floor. For the hole-board experiments, each animal was placed in the center of the hole-board and allowed to freely explore the apparatus for 5 min. The number of head-dips in the holes were recorded. Diazepam (1mg/kg i.p.), an anxiolytic drug was used as a reference drug.
Forced swimming test
The forced swimming test (FST) was performed according to the procedure described Porsolt with slight modifications. Briefly, the animals were individually forced to swim in a transparent glass vessel (25 cm high, 15 cm in diameter) filled with (12.5 cm high) water at 21–24 °C. The duration of immobility (in second) was measured for 5 min. ‘Immobility’ was defined as floating and treading water just enough to keep the nose above water. The immobility reflected a state of lowered mood in which the animals had given up hope of finding an exit and had resigned themselves to the experimental situation. The water was changed after every other trial. Fluoxetine (10mg/kg i.p) was used as positive control drug.

Biochemical estimation
The mice in the experimental group and in control after the behavioral test were sacrificed quickly by using decapitor. The brains were rapidly removed, cerebral cortex and mid brain were then separated out. They were washed quickly with saline, blotted between two damp filter papers, then weighted using electronic balance. Weighed tissue was homogenized with 0.05M phosphate buffer in a cool environment and centrifuged at 10,000 rpm for 15 min for catalase assay and another known weight of tissue was homogenized in 0.32M sucrose, centrifuged at 12,000 rpm for 20 min for lactate dehydrogenase. Protein content was assayed using bovine serum albumin as a reference standard. The enzyme activity was expressed in units/min/mg protein.

Transmission electron microscopy
A specific portion of the cerebral cortex was carefully transferred to glutaraldehyde for transmission electron microscopy by following the standard protocol.

Statistical analysis
The data presented as Mean±SEM. The difference between groups was evaluated by ANOVA which was followed by Turkey multiple comparisons test.

RESULTS
Locomotor activity
In animals pretreated with the extract, the locomotor activity when compared with the control was significantly decreased for 20mg/kg (P<0.05) and for 40mg/kg (P< 0.001). Diazepam at 2mg/kg also suppressed the locomotor activity to a greater extent (P< 0.001) [Fig.1].

Hole-board test
At both the doses of treatment viz, 20mg/kg and 40mg/kg, there is decrease in the number of head dips as compared with the control. The animals that received diazepam (1 mg/kg i.p) significantly increased the number of head dips from that of the control animals (P<0.001) [Fig.2].

Forced swimming test
A significant reduction in immobility time was observed in the mice treated with fluoxetine at 10 mg/kg (P< 0.05). Moreover, the immobility time of the mice treated with AEDSL was increased significantly as compared with the control (P< 0.05 for 20mg/kg, P< 0.01 for 40mg/kg) [Fig.3].

Catalase assay
The level of antioxidant enzyme, catalase was reduced (P<0.05 for 20 mg/kg and P<0.01 for 40 mg/kg) [Fig.4].

Statistical analysis
The data presented as Mean±SEM. The difference between groups was evaluated by ANOVA which was followed by Turkey multiple comparisons test.
Lactate dehydrogenase assay

Administration of AEDSL increased the level of the lactate dehydrogenase significantly \( p<0.05, p<0.01 \) for 20mg/kg and \( p<0.01 \) for 40 mg/kg\[ Fig.5\].

Electron microscopic analysis

For evaluation of the ultrastructure of cerebral cortex cells, transmission electron microscopic (TEM) micrographs of both the treated and control mice were examined \[Fig.6 & Fig.7\].

DISCUSSION

It has been reported that parts of the plant \textit{D}stramonium although possessing medicinal properties, are found to be poisonous if ingested by humans or livestock\[13]. The present work represents a step towards the understanding of the effects of AEDSL on the central nervous system by observing the behavioral parameters viz, locomotor activity, hole-board test, forced swimming test & biochemical parameters viz, CAT, LDH activities and ultrastructural changes in the mice brain. The locomotor activity is a measure of the level of excitability of the CNS\[20\] and decrease of this activity may be closely related to sedation\[20\]. The key factors which plays crucial role in animal behavior are the interactions between neurotransmitters and receptors and it is believed that most behaviors require the integrated activity of many components of the nervous system\[21\]. The AEDSL at both doses of 20mg/kg and 40 mg/kg significantly reduced locomotion relative to control. In order to assess the anxiolytic activity, we used hole-board test, in which number of head dips is gradually inhibited by anxiety. This test has been accepted as an experimental model for the evaluation of psychotinic, sedative and anxiety condition\[22\] and exploratory behavior in animals\[22\]. The anxiolytic agents have been shown to increase the number of head dips\[24\]. A decrease in number of head dips suggests a sedative behavior\[25\]. Diazepam, a known anxiolytic drug significantly increase the number of head dips\[24\]. A decrease in number of head dips suggests a sedative behavior\[25\]. Diazepam, a known anxiolytic drug significantly increase the number of head dips\[24\].

Electroencephalogram (EEG) and evoked potentials were also studied to assess the anxiolytic activity of AEDSL. Both EEG and evoked potentials showed significant decrease in both treated groups \[20\% & 40\%\] which decreased the immobility time significantly. The administration of the plant extract at concentration \( 20 \) mg/kg and \( 40 \) mg/kg was found to induce neurotoxicity in the cerebral cortex as observed in the electron photomicrographs which showed damaged and ruptured mitochondria and non-uniform broken nuclear membrane in the two experimental groups when compared with the control group. A cross section of the brain of rats injected with 50mg/kg and 100mg/kg body weight of \textit{A}torta extract displayed mild fibrosis, nuclear eosinophilia and chromatolysis\[25\]. Exposure to AlCl\(_3\) causes histopathological lesions in cerebral cortex including neuronal degeneration as cytoplasmic vacuolization hemorrhage, precellular edema and gliosis\[20,23\]. The neurotoxicity of AEDSL in this study showed a dose dependent damage. Oxidative stress leads to cellular damage and this effect can be related to low level of antioxidant defense system such as catalase\[21\]. The brain has been reported to be particularly sensitive to oxidative damage due to the high level of lipid content and high metabolic rate\[21\]. It is extremely susceptible to highly reactive oxygen free radicals\[21\]. These free radicals generated cause damage involving cascade of biochemical events leading to neurodegeneration and cell death\[26\]. Catalase is a ubiquitous antioxidant enzyme found in all known organisms and it provides protection against oxidative stress\[26\]. It catalyzes the breakdown of \( \text{H}_2\text{O}_2 \) to \( \text{H}_2\text{O} \) and molecular oxygen\[26\]. The lower level of antioxidant enzymes makes brain more vulnerable to degeneration\[27\]. A decline in this enzyme activity was observed in this study which is consistent with increased free radical production and at the same time makes the tissue more susceptible to biochemical injury. In our study we found elevation of LDH activity after administration of AEDSL. The extracellular appearance of LDH is an important indicator showing cell damage or cell death\[28\]. \textit{D}stramonium contains many phytochemicals, it is therefore possible that these phytoconstituents are responsible for the various behavioral changes & neurotoxic effects as observed by the changes in enzyme activities and ultrastructural damage to the nerve cells as observed in TEM.
CONCLUSION

On the basis of the results obtained from the behavioral study, ARDS at the doses administered was found to possess CNS depressant and sedative properties. The extract was capable of producing oxidative stress, thereby resulting in damage of brain cells which can be inferred by the decrease in CAT activity and elevation in LDH activity. Moreover the neurotoxicity of the cerebral cortex was observed in the TEM photomicrographs. However, the underlying mechanism(s) of action of the plant extract, needs to be further investigated. Our results present the neurotoxicity of reputed medicinal plant, D.stramonium and thus, serious concerns about its long-term use as drug should be reconsidered. This study emphasizes that the plant products should be used very carefully for medical purposes so that its toxicity can be avoided.

ACKNOWLEDGEMENTS

We thank the University Grant Commission for financial assistance and SAIF-NEHU, Shillong for electron microscopy.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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