



## ADVERSE NEUROPHYSIOLOGIC CHANGES IN ALBINO MICE WITH THE ADMINISTRATION OF A PLANT EXTRACT

### Zoology

**Dr. Chinmoy Choudhury**

Zoology Laboratory, Bodofa UN Brahma College, Assam, India

### ABSTRACT

Plant contains thousands of compounds in its ingredients. It does not imply that all of them show similar characteristics. Some plants has been found to used in medicinal purpose. But in traditional knowledge they are reported to be harmful. Such type of a plant has been taken for evaluate its effect in mammalian model. Here the plant extract showed toxicity against the physiology of mice. The decrease in locomotor activity, increase in immobility of force swim & decrease in specific activity of catalase enzyme clearly emphasize the fact of adverse effect in mammalian physiology with the administration of the plant extract.

### KEYWORDS

medicinal, traditional, mammalian, toxicity, physiology, enzyme.

#### Introduction:

Plants and herbs have been used by man from time immemorial. Plants have been used for various purposes including medicine and food. Recently Scientists have shown interest in this field and are doing various studies to evaluate the neurotoxicity of these useful plants so that they can be safely used by human being. Here an attempt has been made to reveal some harmful effect of a plant extract in mouse with the help of behavioural and biochemical study.

#### Climate of the study area:

In any type of experimental assessment climate of the specific study area is considered as a very important aspect because the diversity and characteristics of the flora & fauna of a particular site are directly related to environmental factors. Here in this survey the study area that has been taken is Kokrajhar district of Assam in north-east India. Physiographically this area is very unique; it forms small hillocks, plains, beels and low-lying flood prone areas. The climate experienced here consists of humid weather alongwith frequent rainfall. The temperature is moderate, ranging from 13<sup>o</sup>C – 35<sup>o</sup>C. The soil type of the district includes alluvium laterite red soil and peat soil. The soil P<sub>ii</sub> ranges from 3.8 – 6. The variations of the ecological factors, i.e. temperature, humidity etc. of the valley contribute to luxuriant growth of plant diversity.

#### Methodologies:

The plant was selected for neurophysiologic study from the survey site. Then behavioural study and biochemical investigations in albino mice brain were done. Standard methods and literature were consulted for all the parameters performed. Extensive field study was conducted in the study area in winter season of 2015. Field data was collected from the traditional knowledge of ethnic people. And few information about meditational use of the plant has been found from previous literature study.

#### Preparation of extract:

The fresh part of selected three plants was collected from the nearest forest belongs to Kokrajhar district of Assam, India. The collected material was shade dried and grinded for extraction. Than the grinded material was dipped in distilled water (100 g in 1 L) for 24 h. The filtrate was oven dried at 40<sup>o</sup>C. The extract was weighed and dissolved in distilled water for desired concentration (Akindele and Adeyemi, 2006).

#### Animal experiments:

The albino mice (25-30g) were collected from Pasteur institute, Shillong. The handling and experiments of animals were according to the rules and guidelines of "BUNB College Animal Ethics Committee". After acclimatization, mice were treated with sub lethal dose of extract for evaluation. The mice were divided into three groups, each containing six mice and were treated for seven days. The first one was considered as control group and treated with distilled water (inter peritoneal). The second and third group of mice were treated with *S. anacardium* (i.p.) extract at two doses of 500 and 1000 mg/kg bw to mice considered as low & high dose.

#### Behavioural study:

For behavioural study there were two another groups of mice taken to which standard drug has been treated. First group was treated by Fluoxetine (20 mg/kg, ip.) and considered for Force swim test. The second group was treated by Diazepam (1mg/kg, i.p.) and Locomotor activity was performed.

**Locomotor activity:** In locomotor activity test Rebai and Djebli, 2008 procedure used. The number of crossed squares was recorded for each mouse per time of 5 min for 20 min investigation. Diazepam was used as positive control drug.

**Force swim test:** This was performed by Porsolt *et al.*, 1977. The mice were judged to be immobile when neither hind limb was moving and slightly hunched forward. The duration of immobility was observed and mice were put for five minutes in glass vessel. Fluoxetine was used as standard drug.

#### Biochemical observations:

After behavioural study the mice were sacrificed and a portion of cerebral cortex was separated from brain and washed with saline. Than the tissue were homogenized and used for following biochemical assay.

**Catalase (EC 1.11.1.6):** This was assayed by the method of Aebi, 1974. Catalase is a common enzyme found in nearly all living organisms exposed to oxygen. It catalyzes the decomposition of hydrogen peroxide to water and oxygen. The brain tissue homogenate was added to H<sub>2</sub>O<sub>2</sub> and product obtained, in which disappearance of H<sub>2</sub>O<sub>2</sub> was measured at 240 nm. The unit of enzyme was measured as u/min/mg protien. It is very important enzyme in protecting the cell from oxidative damage by reactive oxygen species.

**Protein :** Protein was measured by Lowry *et al.*, 1951 for calculating specific activity of the enzymes.

**Statistical Analysis:** All the values are expressed as mean ±se. All the values were evaluated by ANOVA along with Students-Newman Keuls post hoc analysis and Turkeys multiple comparison test (Rebai and Djebli, 2008). The significant level is 0.05.

#### RESULTS:

A plant was selected from anacardiaceae family for this screening depending upon the field information and literature study. The collected plant was identified by Botanical Survey of India, Shillong Branch. The plant was *Semecarpus anacardium*. According to Nair and Bhide, (1996) and many other studies the plant contained lots of medicinal value. On the other hand, according to ethnic knowledge of the area the leaf and fruit of the plant secretes lot of latex. Latex is poisonous and may produce burning sensation in the skin of man exposed to it.

#### Behavioural study:

**Locomotor activity test-** The mice administered with *Semecarpus anacardium* extracts showed decreased locomotor activity than

control group (Fig 01). Here low dose group showed significant decrease in locomotion of the test bearing P value <0.05 against control. Similarly the high dose group also found to be highly significant reduction (P value <0.01) compared with control. Diazepam treated group developed a significant decrease in number of square crossed, having P value <0.01 against control.

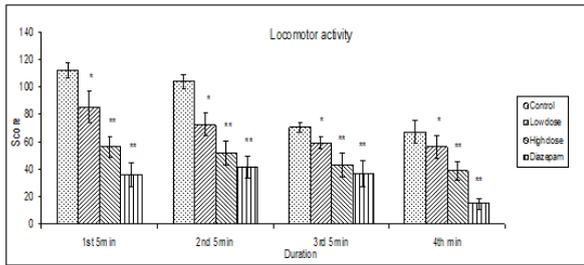


Fig -29: - Histogram (Mean±SE, N=6) of no. of square crossed in locomotor activity test. Here one way ANOVA, SNK-Turkey post hoc test used to analysis the data. (\*P<0.05 & \*\*P≤0.01 vs Control)

**Force swim test:** The mice administered with *Semecarpus anacardium* extracts showed increased immobility time than control group (Fig 02). Here low dose group showed significant elevation in force swim test bearing P value <0.05 against control. Similarly the high dose group also found to be highly significant increased (P value <0.01) compared with control. Fluoxetine treated group developed a significant decrease in immobility time, having P value <0.05 against control.

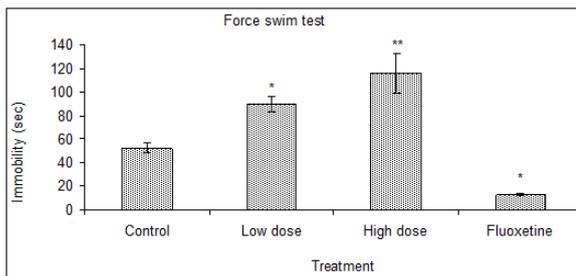


Fig -27: - Histogram (Mean±SE, N=6) of immobility time in Forced swimming test. Here one way ANOVA, SNK-Turkey post hoc test used to analysis the data. (\*P<0.05 & \*\*P≤0.01 vs Control)

#### Enzyme assays:

**Catalase activity-** The mice administered with *Semecarpus anacardium* extracts showed decreased Catalase activity than control group in cerebral cortex (Fig 03). Here low dose group showed significant decrease in catalase of the test bearing P value <0.05 against control. Similarly the high dose group also found to be highly significant reduction (P value <0.01) compared with control.

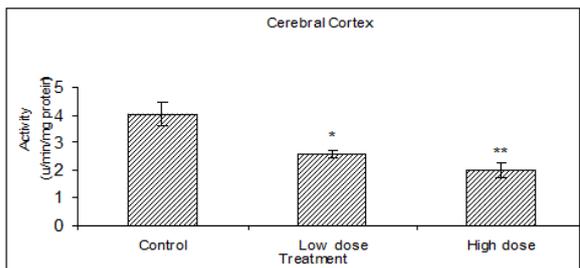


Fig -34: - Histogram (Mean±SE, N=6) of Catalase activity in cerebral cortex. Here one way ANOVA, SNK-Turkey post hoc test used to analysis the data. (\*P<0.05 & \*\*P≤0.01 vs Control)

#### DISCUSSION:

The plant extracts were studied for its effect by performing various behavioural and biochemical experiments-

**Locomotor activity:** It is considered as an index of alertness and a decrease in it, is indicative of sedative activity. The extract of the plant

showed significant decrease in locomotor activity in both the doses. Sedation is the result of central nervous system depression (Ozturk, *et al.*, 1996). Similar result of CNS depression due to sedation when showed as decrease in locomotor activity has been reported by Musa *et al.*, (2006)

**Forced swim test:** In various animal models, the forced swim test (FST) remains one of the most used tools for screening antidepressants (Demouliere *et al.*, 2005). In this study the plant extract (*S. anacardium*) treated mice showed significant increase in immobility time as compared to control mice. The standard drug Fluoxetine significantly decreased the immobility time in the mice. Increase immobility reflects the depressant activity of the extract (Porsolt *et al.*, 1977). Rebai and Djebli (2008) reported that chronic exposure to aluminum chloride increased immobility time in mice. When mice are forced to swim in an unfamiliar environment they tend to become immobile after initial vigorous activity. In this situation the mice loses the hope of escape from the water. This increase in immobility time shows depression in animals.

**Catalase activity:** Oxidative modifications have been proposed as one biochemical change that could lead to the neuropathology, neuronal dysfunction and death (Siedlak, 2009). The excess production of free radicals damages the brain (Finkel and Handbook, 2000). In this study it was found that there is significant decrease in catalase activity in *S. anacardium* treated mice in the regions of brain (Cerebral cortex). Similar result was found by Vani *et al.*, (2000) where catalase activity was decreased due to fluoride accumulation in brain of albino mice. Ward *et al.*, (2001) also reported that brain catalase activity was reduced due to chronic administration of Taurine in rat brain.

#### SUMMARY:

*Semecarpus anacardium* has found to be neurotoxic in our study. It was found that the plant creates CNS depression and anxiety in mouse model. Besides oxidative stress in the mice brain was also reported. The present study emphasizes that the plant studied namely *Semecarpus anacardium* possess adverse neurobehavioural changes in mice. This is a cause of concern as the plants are widely used for medicinal preparations. It is important to use these plants carefully by making proper processing so that its toxicity can be avoided. This requires further study so that detail mechanisms by which the plants causes neurotoxicity can be determined.

#### References:

- Aebi, H. (1974) Catalase. In: Bergmeyer, H. V. (Ed.). Methods of enzymatic analysis. Verlag Chemie Basel, 673-684.
- Akidele, A. J. and Adeyemi, O. O. (2006) Analgesic activity of the aqueous leaf extract of *Byrsocarpus coccineus*. Niger. J. Health. Biomed. Sci. 5 (1): 43-46.
- Demouliere, B. P., Chanu, F. and Bourin, M. (2005) Force swimming test in mice: a review of antidepressant activity. Psychopharmacology, 177:245-255.
- Finkel, T. and Handbook, N. J. (2000) Oxidants, oxidative stress and the biology of ageing. Nature, 408: 239.
- Lowry, O. H., Rosebrough, N. J. and Forr, A. L. (1951) Protein measurement with the folin phenol reagent. J. Biol. Chem. 193: 265-275.
- Musa, A. M., Yaro, A. H. and Danjuma, N. M. (2006) Preliminary phytochemical screening and central nervous system depressant activity of the stem bark of *Ficus thonningii* Blums. Biol. Environ. Sci. Trop. J. 3 (2): 1-6.
- Nair, A. and Bhide, S. V. (1996) Antimicrobial properties of different parts of *Semecarpus anacardium*. Indian drugs, 33: 323-328.
- Ozturk, Y. S., Aydin, R. B., Baser, K. H. C. and Berberoglu, H. (1996) Effect of *Hypericum pericum* L. and *Hypericum calycinum* L. Extracts on the central nervous system in mice. Phytomed. 3 (2): 139-146.
- Porsolt, R. D., Anton, G. and Blaver, N. (1977) Behavioral despair in mice: a primary screening test for antidepressants. Arch. Int. Pharmacodyn. Ther. 36: 229-327.
- Porsolt, R. D., Anton, G. and Blaver, N. (1977) Behavioral despair in mice: a primary screening test for antidepressants. Arch. Int. Pharmacodyn. Ther. 36: 229-327.
- Rebai, Q. and Djebli, N. E. (2008) Chronic exposure to Aluminium chloride in mice: Exploratory behaviours and spatial learning. Advances in Biological Research. 2 (1-2): 26-33.
- Samson, A., Adzu, B., Binda, L. And Wambebe, C. (2001) Neuropharmacological effect of the aqueous extract of *Sphaeranthus senegalensis* in mice. J. Ethnopharmacol. 78: 33-37.
- Siedlak, S.L., Casadesu, G., Webber, K. M., Pappolla, M. A., Atwood, C. S., Smith, M. A. and Perry, G. (2009) Chronic antioxidant therapy reduces oxidative stress in a mouse model of Alzheimer's disease. Free Radic Res. 43(2): 156-164.
- Vani, M. L. and Reddy, K. P. (2000) Effects of fluoride accumulation on some enzymes of brain and gastrocnemius muscle of mice. Research Report. 33 (1): 17-26.
- Ward, R. J., Kest, W., Bruyeer, P., Lallemand, F. and Witte, P. D. (2001) Taurine modulates catalase, aldehyde dehydrogenase and ethanol elimination rats in rat brain. Alcohol & Alcoholism. 36 (1): 39-43.