Neuropharmacological Assessment of Sweet Potato Proteins in Mice

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ABSTRACT
Objective: To assess the neuropharmacological effects of *Ipomoea batatas* [L.] Lam tuberous proteins in male white albino mice.

Subjects: 72 adult male mice of NMRI strain (weighing 25-30 gms) were used. Animals were divided into 12 groups (6 in each group). Each group of animal was treated individually with saline water (5 ml/Kg, p.o.), proteins isolated from *I. batatas* (1.5 and 3.0 mg/Kg, p.o.), standard drugs Diazepam (5.0 mg/Kg, i.p.) and Morphine (5.0 mg/Kg, i.p.).

Methodology: I) General behavior was assessed by a) Undisturbed observation (awareness, alertness, spontaneous activity). b) Response by least provoking stimuli (sound, touch, and pain). Pain nociception determined by small artery clamp at the base of tail & pain anti nociception by tail immersion test. II) Exploratory behavior was determined by Hole Board test.

Statistical analysis: Statistical analysis of difference between groups was evaluated by One way ANOVA followed by post hoc Tuckey test for comparison between drugs (standard and test) and vehicle treated control groups.

Results: The results revealed that the proteins isolated from *I. batatas* (1.5 and 3.0 mg/Kg, p.o.) caused no significant change in exploratory behavior (*p* > 0.05), but demonstrated decrease in spontaneous motor activity, pain response and touch response in general behavior profile.

Conclusion: *I. batatas* tuber proteins have exerted CNS depressant and analgesic activities in the tested animal model.

Key words: *Ipomoea batatas* proteins, mice, general behavior, exploratory behavior, antinociception.

INTRODUCTION

Interest in medicine derived from herbs is growing nowadays because of its health beneficial properties. Sweet potato (*Ipomoea batatas* [L.] Lam) is actually a perennial, viney plant that is widely cultivated as an annual plant in the tropics, where it is grown for its edible tubers. These tubers contain storage reserves laid down by the plant and are utilized as a vital source of nutrition in developing countries. Sweet potatoes tubers are rich in complex carbohydrates, dietary fibers, beta carotene (a vitamin A equivalent nutrient), vitamin C, Vitamin B6 and vitamin E. Low amount of proteins and fat are also present along with copper, manganese, potassium, iron etc.⁠¹ Research has reported health beneficial properties of *Ipomoea batatas* in past. It was reported that *I. batatas* fibers, crude extracts and anthocyanins played an
important role in stabilizing plasma glucose as well as cholesterol levels in animals and humans, growth inhibition of several human colon carcinoma cell lines, in wound healing, and suppress the development of atherosclerotic lesions in mice. Tubers contain proteins, which exhibit antioxidant, antimicrobial and trypsin inhibitor activities.

*I. batatas* is a traditional counterpart and its consumption by human beings is considered to be safe. Food may contain toxic compounds with small safety margins between intake and obvious toxic effect level. Therefore, careful assessment of useful components isolated from edible plants must be carried out. So far neuropharmacological effects of *I. batatas* proteins have not been explored. Therefore present study is designed to assess the effects of *I. batatas* proteins on central nervous system of mice through behavioral studies (gross & exploratory) and by evaluating analgesic activity in these animals.

**MATERIALS AND METHODS**

**Animals**

Adult male mice of NMRI strain (25-30g) were obtained from Department of Pharmacology, Faculty of Pharmacy, University of Karachi. These animals were maintained at controlled temperature (23°C ± 1°C) with 12 hrs. dark/light cycles and given free access to standard food and water ad libitum. Animals were divided into 12 groups (6 in each group). Each group of animal was treated individually with saline water (5 ml/Kg p.o.), proteins isolated from *I. batatas* (1.5 and 3.0 mg/Kg, p.o.), standard drugs Diazepam (5.0 mg/Kg i.p.) and Morphine (5.0 mg/Kg i.p.). Each animal was used once. All these experiments were performed in accordance with the guidelines of the National Institute of Health (NIH).

**Drugs/Chemicals**

Injection Diazepam (Sigma) was used as the standard drug in general behavior profile and exploratory behavior in mice. Injection Morphine was used as the standard drug in tail flick test. Other chemicals used for extract preparation and protein estimation were Tris-HCl buffer (pH 7.5, Scharlau), solid ammonium sulfate (Scharlau), 10 % polyacrylamide gel (SERVA), coomassie brilliant blue stain (BioRad) and Bradford reagent (BioRad).

**Plant material and extract preparation**

The tubers of *I. batatas* were collected in the month of February from Pharmacognostic Garden, Research Institute of Pharmaceutical Sciences, University of Karachi. Tuber tissues were homogenized in 50 mM Tris-HCl buffer (pH 7.5, Scharlau). These homogenates were centrifuged at 10,000 x g for 10 min. The supernatants were brought to 35 % saturation with solid ammonium sulfate (Scharlau) and then resulting precipitate was collected by centrifugation at 10,000 x g for 10 min. 11 The *I. batatas* proteins were further purified by Batch adsorption technique.12-13 and precipitated with ammonium sulfate as described earlier. All conditions were kept as cold as possible (temperature range between +0S°C to +4°C). Sample was suspended in distilled water just before use.

**SDS-PAGE**

SDS-PAGE was performed as described by Laemmli.14 Protein samples were resolved by 10 % polyacrylamide gel (SERVA) under reducing conditions. Proteins were then visualized by
coomassie brilliant blue staining (BioRad).

**Protein estimation**

Protein concentration was determined according to the method of Bradford; bovine serum albumin (SERVA) was used as standard.

**General behavior**

Evaluation of general behavioral profile was performed by the method of Irwin and Dixit et al. Experimental mice (6 in each group) were tested by *I. batatas* protein extracts (1.5 and 3.0 mg/Kg, p.o.), standard drug Diazepam (5.0 mg/Kg, i.p.) and saline water (5 ml/Kg, p.o.) as a vehicle for control group of mice. After drug/vehicle administration, each group of mice was placed individually into the observation cage and observed at 30 min. intervals in the first one hour and at hourly intervals for the next 4 hours. The behavioral activities and changes were observed carefully in each mouse. In order to examine any toxic effect or mortality, animals were kept in observation for 15 days.

_Awareness, alertness and spontaneous activity:_ The awareness and alertness was recorded by visual measure of the animal’s response when placed in a different position and its ability to orient itself without bumps or falls. The normal behavior at resting position was scored as (-), little activity (+), moderate flexibility (++), strong response (+++) and abnormal restlessness as (+++-). The spontaneous activity of the mice was recorded by placing the animal in a bell jar. It usually shows a moderate degree of inquisitive behavior. Moderate activity was scores as (+++) and strong activity as (+++). If there is little motion, the score was (+), while if the animal sleeps, the score was (−). Excessive or very strong inquisitive activity like constant walking or running was scored as (+++). A similar test was performed with the same scoring, when the animals are removed from the jar and placed on a table.

**Sound response:**

Albino mice normally utter no sound, so that vocalization may indicate a noxious stimulus.

**Touch response:**

The touch response was recorded by touching the mice with a pencil or forceps at the various part of the body (i.e. on the side of the neck, abdomen and groin).

**Pain response:**

The pain response was graded when a small artery clamp was attached to the base of the tail, and response was noted.

**Exploratory behavior (Hole-board test)**

The hole-board test was performed as described by Clark et al. White wooden box, with 16 equidistant holes was used. Animals (6 in each group) were treated with *I. batatas* proteins (1.5 and 3.0 mg/Kg, p.o.), standard drug Diazepam (1.0 mg/Kg, i.p.) and saline water (5.0 ml/Kg, p.o.) as a vehicle for control group of mice. After 30 min. of drugs/vehicle administration, experimental mice were placed individually into the center of the box and allowed to move freely inside. The number of times, each mouse pushed its head completely through one of the holes was recorded for the period of 5 min.

**Warm water tail immersion assay**

Male albino mice were devided into four groups of 6 animals each. After stimulating thermal nociceptors, time dependant analgesic activity of *I. batatas* protein extract (1.5 and 3.0 mg/Kg, p.o.) and morphine (5.0
mg/Kg, i.p.), was determined and this activity was compared with saline (5.0 ml/Kg, p.o.) treated group. Antinociception was evaluated by measuring response latencies in the warm water (55°C ± 1°C) tail immersion assay. Response latencies were measured as the time required by the animal to respond to the thermal stimuli. Mice were not permitted to exceed 10 sec. of exposure to the thermal source to prevent prolonged painful stimulation or tissue damage. Base line tail flick latencies were determined prior to any treatment. Antinociception response was evaluated 30 min. after administration of drugs or vehicle and every 30 min. for 2 hrs.

**Statistical analysis**

All these results are presented as mean ± SEM. Statistical comparisons were made by means of one-way analysis of variance (ANOVA) followed by post hoc Tuckey test for comparison between drugs (standard and tested) and vehicle treated groups. Differences between experimental groups were considered statistically significant when \( p < 0.05 \).

**RESULTS**

**SDS-PAGE**

**General behavior**

SDS-PAGE showing ~25 KDa protein isolated from *I. batatas* (L.) Lam tuber (Right side) and protein ladder (Left side). SDS-PAGE was performed in reducing conditions. Test protein is indicated by line marker. In the general behavior test, the group treated with *I. batatas* tuber protein exhibited passivity, mild decrease in spontaneous motor activity, mild decrease in reactivity to startle response, moderate depression of touch and pain responses but produced no influence on awareness and alertness as compared to those in the control group.

Symptoms of altered general behavior subsided after 1½ hr. of treatment. No mortality was recorded up till 15 days of observation of experimental mice. The results of the general behavior test are summarized in Table 1.

**Table 1:** Effect of *I. batatas* protein extract on general behavioral profiles in mice

<table>
<thead>
<tr>
<th>Behavior type</th>
<th>Protein Extract 1.5 mg/Kg</th>
<th>Protein Extract 3.0 mg/Kg</th>
<th>Diazepam 5.0 mg/Kg</th>
<th>Saline water 5.0 ml/Kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneous activity</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>Alertness</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>Awareness</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>-</td>
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<tr>
<td>Sound response</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>Touch response</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>Pain response</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>-</td>
</tr>
</tbody>
</table>

General behavioral test was performed on 4 groups of mice (6 animals in each group), -, no effect; +, slight depression; ++, moderate depression; ++++, strong depression; ++++, very strong depression.

**Exploratory behavior (Hole-board test)**

In the hole-board test, *I. batatas* test proteins demonstrated no significant difference in the number of head dips when compared with the control group \( (p > 0.05) \). However standard drug Diazepam showed significant increase in head dips at dose that did not produce sedation compared with the control and *I.*
**DISCUSSION**

In past, research has been conducted on safety assessment of *I. batatas* tuberous plant and previous reports have demonstrated that terpenoids, isolated from stressed *I. batatas* produced toxic effects. The acute toxicity of ipomeamarone (IPM), a phytotoxin isolated from the injured *I. batatas* tubers was also evaluated in albino rats. Therefore, it is very important to assess safety and toxicity of any therapeutically useful component isolated from this edible plant. In present study, general and exploratory behavior tests were performed on experimental mice for neuropharmacological assessment and safety evaluation of the protein extracts prepared from *I. batatas* tubers. These tests are classical screening of activities on central nervous system of animal models and provide information about anxiety and psychomotor performance. Our findings revealed that the *I. batatas* protein influences the general behavior profile and produced moderate reduction in spontaneous motor activity. Depression of parameters in general behavior of mice suggests central nervous system depressant action and potential sedative effect of test sample.

In the exploratory behavior test (hole-board test), a useful tool for evaluating changes in various emotional states of animals, anxiolytics have been shown to increase the parameter of head dips and decrease in head dips reveals a sedative behavior. Mice treated with *I. batatas* protein extract showed nonsignificant difference in head-dips when compared with the control group indicating no anxiolytic or anxiogenic/sedative effect of sample proteins. In spite of moderate decrease in spontaneous motor activity, test proteins did not alter exploratory behavior of mice in head dip test. Perhaps *I. batatas*...
protein extract has no effect on emotional depression or perhaps the doses of test extract in our study were not sufficient to alter this parameter as it is in the case of benzodiazepine, which acts as an anxiolytic at low doses and produce sedation at higher doses. No mortality was observed up to 15 days after experiment in all treatment groups.

Up till now, very few proteins, either synthetic or natural, have known analgesic property. In the tail immersion assay, proteins obtained from I. batatas tubers presented significant analgesic activity after stimulating thermal nociceptors as compared to control group. Our results demonstrated that our test sample is more potent than morphine. The behavioral tests and analgesic activity here employed, however do not allow discerning the underlying mechanism of action of I. batatas protein extract. Further research is required to clarify the mechanism of central activity of test sample.

CONCLUSION

I. batatas proteins possess CNS depressant and analgesic properties. Further studies are required to explore the underlying mechanism responsible for producing these effects to evaluate the toxicity and safety profile of this tuber protein.

ACKNOWLEDGEMENTS

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REFERENCES


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