ORIGINAL ARTICLE

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 NMRIstrain (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

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Received: April 21, 2009: accepted: November 11, 2009

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.[2-6] Tubers contain proteins, which exhibit antioxidant, antimicrobial and trypsin inhibitor activities. [7-10]

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 [15]; 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.*[16, 17] 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 the 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. [18]

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*. [19] 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. [20] 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

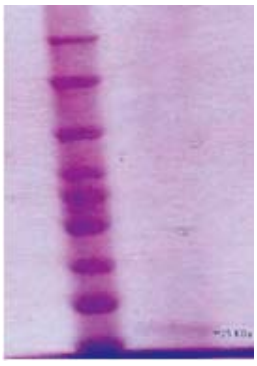

Figure – 1

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

Behavior type	Protein Extract 1.5 mg/Kg	Protein Extract 3.0 mg/Kg	Diazepam 5.0 mg/Kg	Saline water 5.0 ml/Kg
Spontaneous activity Alertness	++	++	++++	-
Awareness Sound response	-	-	+++	-
Touch response Pain response	-	-	+++	-
	+	++	++++	-
	++	++	++++	-
	++	++	++++	-

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.*

batatas protein extract treated groups of mice ($p < 0.05$). The results of the hole-board test are summarized in Table 2.

Table 1: Effect of of I. batatas protein extract in the hole-board test in mice

Groups	Dose	Number of head dips
Saline water (p.o.)	5.0ml/Kg	30.00 ± 0.51
I. batatas test protein (p.o.)	1.5 mg/Kg	29.26 ± 1.50
I. batatas test protein (p.o.)	3.0 mg/Kg	29.16 ± 2.23
Diazepam (i.p.)	1.0 mg/Kg	45.4 ±1.10*

Hole board test was performed on 4 groups of mice (6 animals in each group), * $p < 0.05$ as compared to control (ANOVA was followed by Tukey's post hoc test). Data are reported as mean ± SEM for the n=6 in each group where n = number of animals in each group.

Warm water tail immersion assay

I. batatas protein extract and morphine induced time dependent antinociception and test protein treated group of mice experience a significantly greater delay in tail withdrawal than their control counterparts (Table 3). Thus analgesic response of test protein was significantly different from control group ($p < 0.05$).

Table 1: Effect of of I. batatas protein extract in the hole-board test in mice

Groups	Dose	Tail immersion test (reaction time, seconds)
Saline water (p.o.)	5.0 ml/Kg	1.5 ± 0.28
I. batatas protein extract (p.o.)	1.5 mg/Kg	7.9 ± 0.71*
I. batatas protein extract (p.o.)	3.0 mg/Kg	8.4 ± 0.32*
Morphine (i.p.)	5.0 mg/Kg	4.6 ± 0.24*

Warm water tail immersion test was performed on 4 groups of mice (6 animals in each group), * $p < 0.05$ as compared to control (ANOVA was followed by Tukey's post hoc test. Data are reported as mean ± SEM for the n=6 in each group where n = number of animals in each group.

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. [21-23] 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. [24-25]

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 [26-28] and decrease in head dips reveals a sedative behavior. [28] Mice treated with *I. batatas* protein extract showed non-significant difference in head-dips when compared with the control group ($p > 0.05$) 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.[29] 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. [30-31] 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

Authors are grateful to the Faculty of Pharmacy, University of Karachi for financial assistance.

REFERENCES

1. USDA National Nutrient Database for standard Reference, Release 18, 2005.

2. Suzuki T, Tada H, Sato E, Sagae Y. Application of sweet potato fiber to skin wound in rat. Biol Pharm Bull 1996; 19: 977-83.

3. Kaneshiro T, Suzui M, Takamatsu R, Murakami A, Ohigashi H, Fujin T, et al. Growth inhibitory activities of crude extracts obtained from herbal plants in the Ryukyu Islands on several human colon carcinoma cell lines 2005; 6: 353-8.

4. Miyazaki K, Makino K, Iwadate E, Deguchi, Y, Ishikawa F. Anthocyanins from purple sweet potato Ipomoea batatas cultivar Ayamurasaki suppress the development of atherosclerotic lesions and both enhancements of oxidative stress and soluble vascular cell adhesion molecule-1 in apolipoprotein E-deficient mice J Agric Food Chem 2008; 56:11485-92.

5. Kusano S, Abe H. Antidiabetic activity of white skinned sweet potato (Ipomoea batatas L.) in obese Zucker fatty rats. Biol Pharm Bull 2000; 23:23-6.

6. Ludvik B, Neuffer B, Pacini G. Efficacy of Ipomoea batatas (Caiapo) on diabetes control in type 2 diabetic subjects treated with diet. Diabetes Care 2004; 27:436-40.

7. Yeh K-W, Chen JC, Lin MI, Chen Y, Lin C. Functional activity of sporamin from sweet potato (Ipomoea batatas Lam): a tuber storage protein with trypsin inhibitory activity. Plant Mol Biol 1997; 33: 565-70.

8. Hou W-C, Lin Y-H. Dehydroascorbate reductase and mono dehydroascorbate reductase activities of trypsin inhibitors, the major sweet potato (Ipomoea batatas [L.] Lam) root storage protein. Plant Science 1997; 128: 151-8.

9. Hou WC, Chen YC, Chen HJ, Liu YH, Yang LL, Lee MH. Antioxidant activities of a 33 KDa root storage protein of sweet potato (Ipomoea batatas [L.] Lam cv. Tainong 57). J Agric Food Chem 2001; 49:2978-81.

10. Huang GJ, Lai HC, Chang Ys, Sheu MJ, Lu TL, Huang SS et al. Antimicrobial dehydroascorbate reductase, and monodehydroascorbate reductase activities of defensin from sweet potato [Ipomoea

batatas (L.) Lam. 'Tainong 57'] storage roots. Agnic Food Chem 2008; 56: 2989-95.

11. Colowick SP, Kaplan NO (eds). Methods in Enzymology. New York (NY): Academic Press; 1955. p. 67-90.

12. Bonnerjea J, Jackson M, Hoare M, Dunnill P. Affinity flocculation of yeast cell debris by carbohydrate specific compounds. Enzyme Microb Tech 1988; 10:357-60.

13. Chase HA. Purification of proteins by adsorption chromatography in expanded beds. Trends Biotechnol 1994; 12:296-303.

14. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970; 227:680-5.

15. Bradford MM. A rapid and sensitive method for the quantification of microgram quantities of proteins utilizing the principle of protein-dye binding. Anal Biochem 1976; 72:248-54.

16. Irwin S. In animal clinical pharmacological Techniques in Drug Evaluation; Nordin, JH. Siegler PE Ed. Yambook Medical Publication, Chicago 1962; 36.

17. Dixit VK, Varma KC. Effects of essential oil of leaves of Blumea lacera DC on central nervous system. Indian J Pharmacol 1976; 18:7–11.

18. Turner RA (ed). Screening methods in pharmacology. New York, Academic Press 1965; 26-35.

19. Clark G, Koster AG, Person DW. Exploratory behavior in chronic disulfoton poisoning in mice. Psychopharmacology 1971; 20:169-71.

20. Ghosh MN (ed). Fundamental of experimental pharmacology. 2nd ed. Calcutta, Scientific Book Agency, 1984, p 153.

21. Boyd MR, Wilson BJ. Isolation and characterization of 4-ipomeanol, a lung-toxic furanoterpenoid produced by sweet potatoes (Ipomoea batatas). J Agric Food Chem 1972; 20:428–30.

22. Wilson BJ, Burka LT. Toxicity of novel sequiterpenoids from the stressed sweet potato (Ipomoea batatas). Food Cosmet Toxicol 1979; 17: 353-5.

23. Pandey G. Acute toxicity of ipomeamarone, a Phytotoxin isolated from the injured Sweet Potato. Phcog Mag 2008; 4: 89-92.

24. Ozturk Y, Aydin S, Beis R, Baser KH, Berberoglu. H. Effects of Hypericum perforatum L. and Hypericum calycinum L. extracts on the central nervous system in mice. Phytomedicine 1996; 3:137-44.

25. Perez RM, Perez JA, Garcia LM, Sossa M. Neuropharmacological activity of Solanum nigrum fruit. J Ethnopharmacol 1998; 62:43-8.

26. Tadeka H, Tsuji M, Matsumiya T. Changes in head-dipping behavior in the hole-board test reflects the anxiolytic state in mice. Eur J Pharmacol 1998; 350:21-9.

27. Crawley JN. Exploratory behaviour models of anxiety in mice. Neurosci Biobehav 1985; 9:37-44.

28. File S, Pellow S. The effect of triazolobenzodiazepines in two animal tests of anxiety and on the hole-board. Br J Pharmacol 1985; 86:729-35.

29. Onaivi ES, Maguiri PA, Tsai NF, Davies MF, Loew G. Comparison of behavioral and central BDZ binding profile in three rat lines. Pharmacol Biochem Behavior 1992; 43:825-31.

30. Broccardo M, Erspamer V, Falconieri Erspamer G, Improta G, Linari G, Melchiorri P, et al. Pharmacologival data on dermorphins, a new class of potent opioid peptides from amphibian skin. Br J Pharmacol 1981; 73:625-31.

31. Ivanova VP, Sorochiniskaia EI, Lozhkina TK, Anokhina W. Immunomodulating and analgesic activity of synthetic fragments of various proteins and immunopeptides. Ukr Biokhim Zh 1988; 60:3-9.