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Research article

Neurobehavioral Activity of *Pandanus tectorius* Parkinson (Pandanaceae) Leaf Extract in various Experimental Models

Punitkumar Rameshchandra Bhatt*, Kajal Punitkumar Bhatt

Department of Pharmaceutical Sciences, Saurastra University,Rajkot, Gujarat, India
Correspondence: puns003@gmail.com (+91 9725209039)

Abstract

Pandanus tectorius Parkinson is a medicinal and folklore herb from India belong to family pandanaceae. This herb has been described in scabies, leucoderma, cardiac diseases and brain diseases etc. Hydro alcoholic (HA) extract of leaf was prepared and further fractionated with different solvent with increasing polarity. Ethyl acetate fraction and n-butanol fraction was selected for pharmacological activity. All these three crude extracts were evaluated for elevated plus maze, open field test and forced swim test models and it was found that behavior of the mice in all the models has been improved as compared to standard and control compounds. Phytochemical screening has revealed that n-butanol fraction and ethyl acetate fraction shows presence of flavonoids which could be responsible for the pharmacological action. All the data obtained was analyzed by GraphPad.Prism.v5.0.3.477. Data were expressed as mean ± SEM from 6 animals, The significance of difference in means between control and treated animals for different parameters was determined by using One-way Analysis of Variance (ANOVA) followed by multiple comparisons Dunnett's test. A value of $p<0.05$ was considered statistically significant.

Keywords: Anti-depressant activity, anxiolytic activity, medicinal plant, *Pandanus tectorius*, neurobehavioral

Introduction

Pandanus tectorius Parkinson (Pandanaceae) is commonly known as 'Kewado' in Gujarat region of India has already been studied chemically and found to contain triterpenoids and flavanoids [1]. On the basis of literature survey, triterpenoids is a major constituent of the plant of Pandanus tectorius. Leaves can be used in scabies, leucoderma, cardiac diseases and brain diseases [2].

Present study deals with preparation of crude extract of fresh leaves collected from Junagadh region of Gujarat, authenticated and pharmacognostical and phytochemical study were done. Fresh leaves were subjected to extract with ethanol and water in the ratio of 60:40, along with this, fractionation of HA extract was done with different solvents but only n-butanol and ethyl acetate fraction was selected on the basis of phytochemical screening. Three models were selected named forced swim test, elevated plus maze and open field test used to check behavior of Swiss albino mice.

Materials and Methods

Plant material

Fresh leaves of *Pandanus tectorius* were collected from the ground of 108 ambulance office, Junagadh, Gujarat (India) during the month of September-October 2010. Herbarium was prepared and the specimen was further identified and authenticated by Dr. A. S. Reddy, Associate Professor, Sardar Patel University, Anand, Gujarat (India).

Preparation of plant extract

The extract is also fractionated by shaking with solvents with decreasing polarity like petroleum ether, n-hexane, chloroform, ethyl acetate and n-butanol etc and fraction of each solvent were analyzed for phytochemical screening.

Animals

The albino mice of either sex (35-40 g) were used for study. The animals were housed in a five mice per cage under well controlled conditions of temperature (25±1°C), humidity (55±5 %) and 12h/ 12h light-dark cycle (light on 07.30-19.30 h). Animals had access to standard pellet diet (Pranav Agro Industries Ltd., India) and water given ad libitum. The protocol of the experiment was approved by the institutional animal ethical committee as per the guidance of the Committee for the purpose of Control and Supervision of Experiments on Animals (Protocol approval no.: SU/DPS/IAEC/2011/09). For the present study, animals were randomized into 5 groups of 6 animals each and allowed to acclimatize for 1 week before the experiments.

Acute toxicity study

Acute toxicity study of mice was done as per OECD guidelines. No significant toxicity was found.

Results

Phytochemical analysis

The preliminary phytochemical analysis shows the presence of flavonoids in the HAE and fractionation of the HAE gives nBF and EAF consequently (Table 1).

Table 1. Phytochemical screening of P. *tectorius*

Test	AP. Ether	n-hexane	CHCl3	E.A	n-butanol	HA
Alkaloids	-	-	-	-	-	-
Glycoside	-	-	+	+	+	+
Carbohydrates	+	-	+	-	-	+
Phytosterols	+	+	+	-	+	-
Saponins	-	-	-	+	+	+
Phenolic compounds	-	-	-	-	-	+
Proteins & amino acids	-	-	-	-	-	+
Gums & mucilage	-	-	-	-	-	-
Terpenoids	-	-	-	+	-	+
Flavanoids	-	-	-	+	-	+
Fixed oil and fats	+	+	-	-	-	+

Elevated plus Maze [3]

The ANOVA revealed significant effects of the *Pandanus tectorius* hydro alcoholic extract and fractions treatment on the number of entries into the open arms, ($p<0.001$) and on the time spent in the open arms, ($p<0.001$), of the elevated plus-maze. Ethyl acetate faction gives higher significant result as compare to n-butanol fraction and hydro alcoholic extract (Table 2).

Table 2. Effect of *P. tectorius* hydro alcoholic extract and fractions and DZP on elevated plus maze test in mice

Group	Dose (mg/kg)	No. of entries		Time spent (sec)	
		open arm	close arm	open arm	close arm
Control	Vehicle	1.6 ± 0.33	4.3 ± 0.21	5.00 ± 0.58	295 ± 0.58
DZP	5	4.0 ± 0.33***	1.6 ± 0.76***	176.33 ± 5.11***	123.6 ± 5.11***
HAE	100	2.5 ± 0.30*	2.8 ± 0.30	61 ± 8.79*	238.8 ± 13.94*
	200	3.3 ± 0.47**	2.5 ± 0.47*	162 ± 14.9***	137.5 ± 12.71**
	50	3.6 ± 0.44***	1.8 ± 0.3**	63.83 ± 5.6**	236.16 ± 5.6*
EA fraction	100	3.9 ± 0.57***	1.5 ± 0.34***	166.33 ± 5.20***	133.83±5.20***
n butanol	50	2.1 ± 0.22	3.8 ± 0.21	49 ± 5.32	251 ± 5.32
fraction	100	3.3 ± 0.42*	2.5 ± 0.25*	90.83 ± 4.4**	209.16 ± 4.4**

Values are expressed as mean ± SEM (n = 6); One-way Analysis of Variance (ANOVA) followed by multiple comparison Dunnett's test, *$p<0.05$, **$p<0.01$, ***$p<0.001$ vs. Control

Dunnett's post test analysis showed a significant increase of the no. of entries after the doses of 100, 200 mg/kg of hydro alcoholic extract and 50, 100 mg/kg of fraction. This effect was accompanied by a significant increase in the time spent in the open arms of the maze after the doses of 100, 200 mg/kg of extract and 50, 100 mg/kg of fraction. The effects on the open arms exploration elicited by the Ethyl acetate fraction were not significantly different from those observed after diazepam administration (5 mg/kg; i.p.).

Forced Swim Test [4]

The effects of the Pandanus tectorius extract and fractions treatment on active behaviors in the FST of mice are shown in Table 3.

Table 3. Effect of *P. tectorius* hydroalcoholic extract and fractions on forced swim test in mice

Group	Dose (mg/kg)	Immobility Time (sec)
Control	Vehicle	168.8 ± 7.6
Imipramine	12.5	85.33 ± 5.7***
HAE	100	123.8 ± 10.7**
	200	90 ± 7.1***
Ethyl acetate	50	104.2 ± 9.6***
Fraction	100	89.17 ± 6.1***
n-butanol	50	150.7 ± 7.6
fraction	100	132 ± 7.06*

Values are expressed as mean ± SEM (n=6); One-way Analysis of Variance (ANOVA) followed by multiple comparison Dunnett's test, *$p<0.05$, **$p<0.01$,***$p<0.001$ vs. Control.

The ANOVA revealed significant effects of treatment on immobility, ($p<0.001$) swimming behavior, Post test analysis demonstrated that the *Pandanus tectorius* extract 100, 200 mg/kg and fractions 50, 100 mg/kg significantly shortened the immobility time in comparison to control values. Ethyl acetate faction gives higher significant result as compare to n-butanol fraction and hydro alcoholic extract. The effects on the immobility time elicited by the ethyl acetate fraction were not significantly different from those observed after imipramine administration (12.5 mg/kg; i.p.).

Open Field Test [5-6]

The effects of the *Pandanus tectorius* extract and fractions treatment on the number of line cross and no. of rearing on mice are shown in Table 4.

The ANOVA revealed significant effects of treatment on the number of square cross and number of rearing ($p<0.001$), Post test analysis demonstrated that the *Pandanus tectorius* hydro alcoholic extract

Table 4. Effect of P. tectorius hydro alcoholic extract and fractions and DZP on open field test in mice

Group	Dose (mg/kg)	No. of line crossing	No. of rearing
Control	-	97.0 ± 3.21	4.0 ± 1.30
DZP	5	34.17 ± 4.56***	1.0 ± 0.63***
HAE	100	61.67 ± 6.21**	1.8 ± 0.85*
	200	41.83 ± 4.21**	1.8 ± 0.51*
Ethylacetate	50	60.50 ± 9.3**	1.5 ± 0.67**
Fraction	100	37.5 ± 6.32***	1 ± 0.33***
n-butanol	50	83.50 ±10.32	3.3 ± 1.04
Fraction	100	63.67 ± 9.68**	2.0 ± 0.89*

Values are expressed as mean ± SEM (n=6); One-way Analysis of Variance (ANOVA) followed by multiple comparison Dunnett's test, *p<0.05, **p<0.01, ***p<0.001 vs. Control

100, 200 mg/kg and fractions 50, 100 mg/kg significantly decrease the number of line cross and number of rearing in comparison to control values. Ethyl acetate faction gives higher significant result as compare to n- butanol fraction and hydroalcoholic extract. The effect on the number of line cross and no. of rearing elicited by the ethyl acetate fraction were not significantly different from those observed after diazepam administration (5 mg/kg; i.p.).

Discussion

The present study was aimed to check neurobehavioral action of extracts and fractions of the *P. tectorius* leaves, a folklore plant from India. Preliminary phytochemical screening confirmed the presence of flavanoid which was the prime target of the study for the pharmacological evaluation.

Forced Swim Test has been validated tool for the evaluation of the drug having antidepressant action [7]. In the present study, oral administration of HAE at doses of 100 and 200 mg/kg were producing significant ($p<0.001$) reduces immobility of mice. EAF at a dose of 50 and 100 mg/kg body weight produced almost similar effect of standard drug (Imipramine 12.5 mg/kg) and produced a more significant effect than HAE and nBF, so it can be assumed that flavonoid fraction of EAF of *P. tectorius* has profound antidepressant action.

Similarly, for anxiolytic property of the drug EPM model has been commonly used. The frequency and time spent in the open arm is the major index in the EPM, given the fact that an open area is extremely averse to rodents [8]. Rodents demonstrate anxiety and fear when placed in a new environment and behavior could be determined through the observation of rearing and grooming [9].

With this hypothesis, present study shown, EAF at the doses of 50 and 100 mg/kg produced significant anxiolytic action ($p<0.001$) when compared to standard drug diazepam at a dose of 5 mg/kg. This may predict that HAE may interact with GABAergic transmission as diazepam and responsible for the anxiolytic action [10].

For evaluation of exploratory behavior OFT has been used. The animal is placed in the center of the arena and the number of rearing and number of square crossed were counted for 5 minutes. Rodent shows fear and curiosity when placed in a new environment. Behavior is observed by calculating the number of squares crossed [11].

EAF of *P. tectorius* produced significant anti anxiety action ($p<0.001$) as compared to standard drug diazepam (5 mg/kg) and other fractions. This suggests that the drug may have anxiolytic action.

Therefore, the present study was prime attempt to explore neuro behavioral action of *P. tectorius* by using basis laboratory models. More work that is precise will do in the future with isolating compounds from plant to justify its traditional claim.

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Conflict of interest

The authors do not have any conflict of interest.

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