

Memory enhancing activity of methanolic extract of *Pterocarpus marsupium* Roxb.

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Received: 23 October 2011, **Revised:** 3 November 2011 **Accepted:** 4 November 2011

Abstract

The present study was undertaken to investigate the effects of methanolic extract of *Pterocarpus marsupium* Roxb. on learning and memory in albino mice. Two doses of methanolic extract of *P. marsupium* (25 and 50 mg/kg p.o.) were administered for neurotoxicity test. The mice administered with 25 mg/kg of the extract could significantly maintain the equilibrium on the rotating rod. Elevated plus-maze and Morris water maze test were employed to test learning and memory in mice. Administration of *P. marsupium* significantly ameliorated scopolamine induced amnesia in elevated plus maze test as indicated by increase in inflexion ratio and reduction in transfer latency. In the Morris water maze test, *P. marsupium* improved the impairment of spatial memory induced by scopolamine as indicated by the formation of reference and working memories. Since scopolamine-induced amnesia was reversed by *P. marsupium*, it is possible that the beneficial effects on learning and memory were due to facilitation of cholinergic-transmission in mouse brain. However, further studies are required to identify the exact mechanism of action. In the present investigation, *P. marsupium* has shown promise as a memory enhancing agent in all the laboratory models employed.

Keywords: *Pterocarpus marsupium*; Memory; Elevated plus maze; Morris water maze; Scopolamine; Piracetam

Introduction

Dementia is a mental disorder characterized by loss of intellectual ability sufficiently severe as to interfere with one's occupational or social activities and it invariably involves impairment of memory. The most common cause of dementia is a progressive neurodegenerative disorder associated with loss of neurons in distinct brain areas. The central cholinergic pathways play a prominent role in learning and memory processes (Nabeshima, 1993). Centrally acting anti-muscarinic drugs (e.g. scopolamine) impair learning and memory both in animals (Higashida and Ogawa, 1987) and human beings (Sitaram *et al.*, 1978). The noot-

ropic drugs belong to the class of psychotropic agents with selective facilitatory effect on intellectual performance, learning and memory (Giurgea, 1973). Since allopathic system of medicine is yet to provide a radical cure, it is worthwhile to look for new directions, which would minimize the memory loss in elderly patients. In the recent years, there has been a phenomenal rise in the interest of scientific community to explore the pharmacological action or to confirm veracity of claims made about herbs in the Ayurveda. Several plants have been reported to possess nootropic activity (Nadkarni, 1976).

The plant *Pterocarpus marsupium* Roxb. belonging to the family Leguminaceae is popularly known as Indian Kino tree or Bijasar or Vijaysar in Hindi. It is commonly grow in the hilly regions of central and peninsular India (Jain, 1968). It has a long history of numerous traditional and ethnobotanical applications in diverse cultures. Many tribes considered it as a cure for all ailments. Bark is used as anti-diabetic (Joshi et al., 2004, Rout et al., 2009, Vats et al., 2002) and as hepatoprotective (Mankani et al., 2005) and also as anti-diarrheal (Grover et al., 2004). Leaves are used in gastrointestinal diseases (Rout et al., 2009). Traditionally stem have been used for the treatment of neurological problems (Acharya et al., 2005) however, there has not been sufficient investigation to establish *P. marsupium*'s biological effects in preventing neuronal effects. The present study was undertaken to investigate the effects of *P. marsupium*, popularly known as Indian kino tree on learning and memory in mice.

Materials and Methods

Plant material

The wood of *P. marsupium* was obtained from a commercial source and authenticated by Dr. H.B. Singh, Scientist F & Head, Raw Materials Herbarium & Museum, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, India. A voucher specimen has been deposited at the NISCAIR Herbarium (NISCAIR/RH-MD/Consult/-2009-10/1313/116 dated November 13, 2009).

Extraction

The powder plant material (500 gm) was extracted with 95 % methanol using Soxhlet extraction apparatus. The solvent was completely removed under reduced pressure till the semi solid mass was obtained. Total 80 gm of dry crude methanol extract was obtained. The yield of methanolic extract was found to be 16 % w/w. The extract was stored in the refrigerator and a weighed amount was suspended in distilled water using Tween 80 (0.2% v/v) as the suspending agent prior to administration.

Animals

Adult Swiss albino mice (20-25 gm) of either sex were procured from breeding unit of Indian Institute of Toxicology Research, Lucknow, U.P, India. The animals were housed in polypropylene cage under standard conditions (25 ± 2 °C, 12 h light and dark cycle) with free access to commercial pellet feed (Ashirwad Industries, Mohali, Punjab) and water *ad libitum*. All the experimental procedures and protocols involving animals were reviewed by the

Institutional Animal Ethical Committee (Registration number: 1279/ac/09/CPCSEA) and were in accordance with the CPCSEA guidelines. All experiments were performed in the morning according to the guidelines for the care of laboratory animals. (Zimmermann *et al.*, 1983).

Phytochemical testing

Preliminary phytochemical screening of *P. marsupium* extract was done to test the presence of the active chemical constituents such as alkaloids, flavonoids, tannins, phenolic compounds, saponins, fixed oils and fats (Khandelwal, 2004).

Acute toxicity study

The methanolic extract of *P. marsupium* was administered orally in dose of 50, 100, 200, 400, 800 and 1600 mg/kg to groups of mice (n = 6) and percentage mortality was noted 24 h later. In our study, final dose was selected on the basis of neurotoxicity study.

Neurotoxicity test

The neurotoxicity test was performed according to the methods of Dunham and Miya, 1957. In this test a knurled rod (2.5 cm in diameter) was rotated at a speed of 10 rev/min. A normal mouse could maintain its equilibrium for longer period. In a drug treated mouse the neurological deficit was indicated by its inability to maintain equilibrium for 1 min in each of three trials. *P. marsupium* was administered orally in doses of 25 and 50 mg/kg and the animals were tested 30 min after the drug administration.

Behavioral study

Elevated plus maze model

The elevated plus-maze consisting of two open arms (16 × 5 cm) and two enclosed arms (16 × 5 × 12 cm) was used. The maze was elevated to height of 25 cm. Mice were placed individually at end of an open arm facing away from central platform and the time took to move from the end of open arm to either of closed arm (Transfer latency, TL) was recorded. If the animal did not enter into one of the enclosed arms within 90 sec, it was gently pushed into one of the two enclosed arms and the TL was assigned as 90 sec. The mice was allowed to explore the maze for another 10 sec and then returned to its home cage. Retention of this learned-task was examined 24 h after the first day trial. Transfer latency after 24 h was expressed as “Inflexion Ratio, IR” using the formula described by Jaiswal and Bhattacharya (1992): $IR = (L_1 - L_0) / L_0$. Where L_0 is the transfer latency after 24 h and L_1 is the initial transfer latency in seconds. Mice of either sex, weight 25-30 gm, were divided into six groups consisting of 6 animals each. Group I: Distilled water (10 ml/kg) was administered orally for 7 days. After 90 min of administration on 7th day transfer latency was recorded. Retention of learned task was examined after 24 h. Group II: Scopolamine HCl (0.4 mg/kg) was injected before training. TL was recorded after 45 min of injection. Retention was examined after 24 h. Group III: Methanolic extract (25 mg/kg) was administered orally for 7 days. TL was noted after 90 min of administration on 7th day and after 24 h. Group VI: stan-

ard group received piracetam (100 mg/kg). TL was recorded after 90 min of administration on 7th day and after 24 h. Group V: received extract with maximum nootropic activity (25 mg/kg) for 7 days and on 7th day after 90 min of extract administration, scopolamine HCl (0.4 mg/kg) was given. TL was recorded after 45 min of injection and after 24 h. Group VI: received piracetam (100 mg/kg) for 7 days and on 7th day after 90 min of extract administration, scopolamine HCl (0.4 mg/kg) was given. TL was recorded after 45 min of injection and after 24 h.

Morris water maze (MWM) model

The MWM task has been used extensively to investigate spatial learning and memory in rodents (Morris, 1984., Bejar *et al.*, 1999, Frick *et al.*, 1995, Gordon *et al.*, 1995). Maze is a semi spherical pool (diameter: 90 cm, height: 45 cm) filled with water to a depth of approximately 30cm. The pool was divided into four equal quadrants and a platform was submerged 1 cm below the opaque surface in the centre of one of the quadrants. The pool was located in a test room and many cues external to the maze were visible from the pool (e.g., pictures, lamps, etc.), which could be used by the rats for spatial orientation. The position of the cues was kept constant throughout the task. The mice were released into the water and allowed for 90 sec to find the platform. Animals received 2 trials per day with 20 minutes inter-trial interval for 4 days, the latency to find the platform was low (< 10 sec). During each trial, the escape latencies of mice were recorded. This parameter was averaged for each session of trials and for each mouse. Once the mouse located the platform, it was permitted to remain on it for 10 sec. If the mouse did not locate the platform within 90 sec, it was placed on the platform for 10 sec and then removed from the pool. The point of entry of the mouse into the pool and the location of the platform for escape remained unchanged between trials 1 and 2 but was changed on each day. The decrease in escape latency from day to day in trial 1 represents long-term memory or reference memory while that from trial 1 to trial 2, represents short-term memory or working memory.

Statistical Analysis

Data obtained by elevated plus maze were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test. $P < 0.05$ was considered as statistically significant. The data obtained by Morris water maze were analyzed using two way analysis of variance (ANOVA).

Results

Phytochemical testing

Phytochemical testing showed that the methanolic extract of *P. marsupium* contains carbohydrates, glycosides, saponins, tannins and flavonoids.

Acute toxicity study

The result of the acute oral administration of methanolic extract of *P. marsupium* in various doses of 50, 100, 200, 400, 800 and 1600 mg/kg indicated no mortality up to 7 days after treatment.

Neurotoxicity test

Mice treated with lower dose of methanolic extract of *P. marsupium* (25 mg/kg) were able to maintain equilibrium on the rotating rod for more than 5 min, whereas the animals treated with 50 mg/kg of extract exhibited motor in-coordination and fall off time was found to be 50.13 ± 1.37 sec.

Elevated plus maze

Transfer latency (TL) of first day reflected learning behavior of animals whereas, TL of second day reflected retention of information or memory. *P. marsupium* (MEPM 25 mg/kg) and piracetam (100 mg/kg) administration for 7 days orally significantly decreased TL on first day as well as second days, indicating significant improvement of learning and memory. Scopolamine (0.4 mg/kg) injected before training impaired learning significantly as indicated by increased TL. Methanolic extract of *P. marsupium* (25 mg/kg) and piraceta-m (100 mg/kg) administered orally for 7 days protected the animals from scopolamine-induced impairment in learning and memory (Table 1).

Morris water maze

The improvement effects of the methanolic extract of *P. marsupium* on special learning and memory process were assessed by Morris water maze test. The escape latency for finding the hidden platform is depicted in Figure 1. The control group mice showed a marked reduction in escape latencies from day 1 to day 4 in first trial. In addition, the result exhibited a significance differences of escape latencies both the 1st and the 2nd trial on day 1st (Fig. 1A). In contrast, escape latencies of the scopolamine-administered mice did not significantly change during the 4 training days in which animals wasted the time exploring the margin of the pool (Fig. 1B). The escape latencies of *P. marsupium* (25mg/kg) and piracetam (100 mg/kg) - treated mice were rapidly decreased after day 1. These groups also exhibited a marked reduction in escape latencies from trial 1 and trial 2 on day 1, and reach-ed stable latencies after day 2 (Fig. 1C-D). The escape latencies for trial 2 were significantly lower than trial 1 for the scopolamine plus MEPM (25mg/kg) and for scopolamine plus Piracetam throughout the study (Figure 1 E-F).

Table 1. Effect of *P. marsupium* extract on transfer latency of mice using elevated plus-maze paradigm.

Groups	TL on 1 st /7 th day	TL after 24 h	Inflexion Ratio
Group I (Distilled water 10 mg/kg)	17.83±0.53	16.16±0.53	0.06±0.53
Group II (Scopolamine 0.4 mg/kg)	37.33±2.57	53.00±2.61	0.29±2.57
Group III (ME PM 25 mg/kg)	7.83±0.34 ^a	6.83±0.53 ^a	0.14±0.23
Group IV (Piracetam 100 mg/kg)	6.83±0.78 ^a	5.60±0.47 ^a	0.21±0.53
Group V (MEPM 25 mg/kg + Scopolamine)	8.66±0.79 ^b	5.50±0.47 ^b	0.57±0.47
Group VI (Piracetam + Scopolamine)	6.83±0.78 ^b	6.00±0.40 ^b	0.13±0.40

Values are mean ± S.E.M.

^a P<0.05 as compared to control group.

^b P<0.05 as compared to scopolamine treated group.

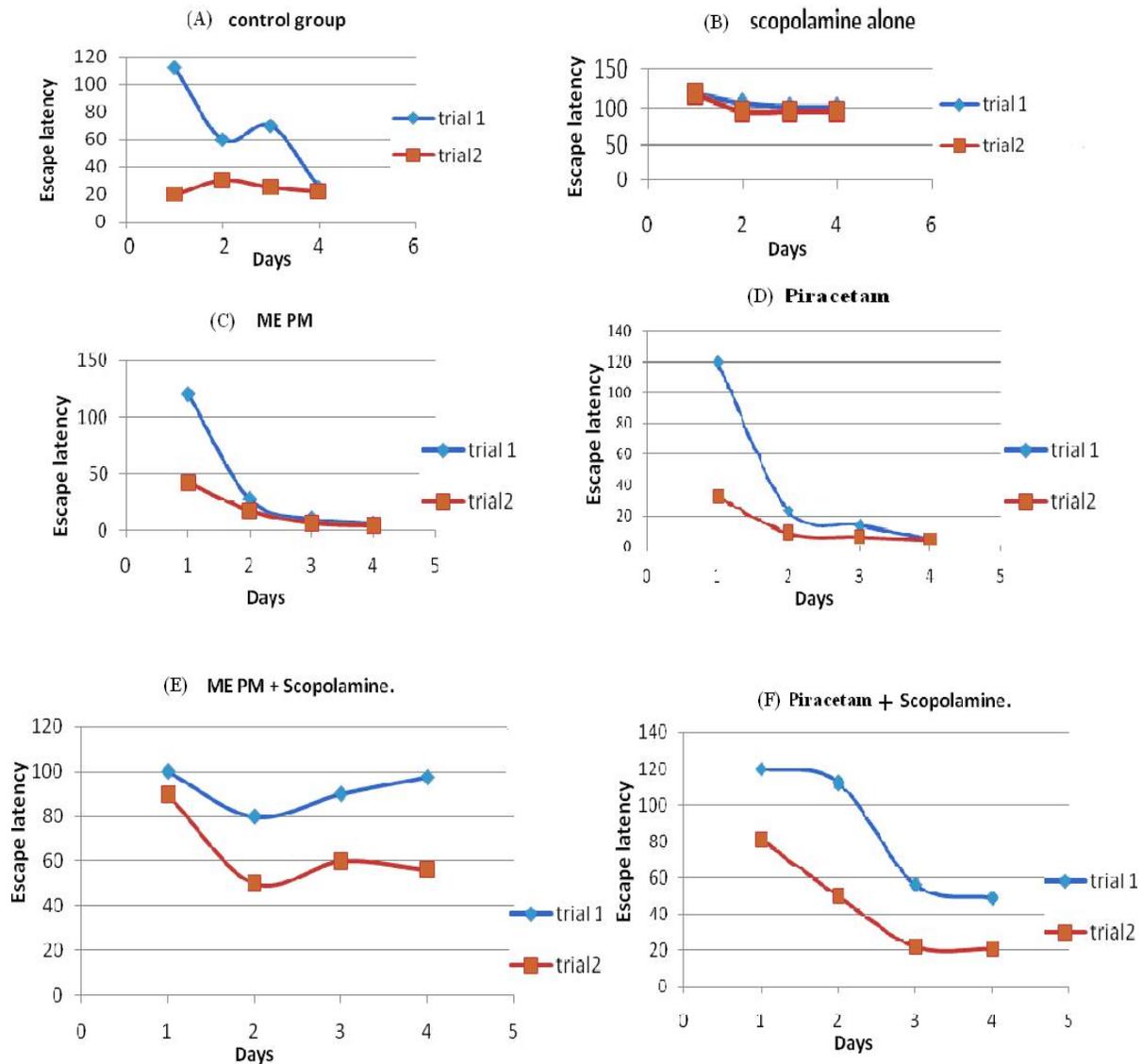


Figure 1. The enhancing effect of acute administration of *P. marsupium* (25 mg/kg) on spatial memory impairment induced by scopolamine in mice. Mice were given two sessions of trials each day for 4 consecutive days. The swimming time required for mouse to escape to platform was recorded in each day. Each day, the mice were treated with *P. marsupium* (25 mg/kg). After 90 min, amnesia was induced by scopolamine (0.4 mg/kg). All mice were tested for spatial memory 30 min after the administration of scopolamine. The values shown are the mean escape latency \pm S.E.M. (A) Distilled water (10 ml/kg) treated group. (B) Scopolamine (0.4 mg/kg) treated group. (C) *P. marsupium* (25 mg/kg) treated group. (D) Piracetam (100 mg/kg) treated group. (E) *P. marsupium* (25 mg/kg) treated group 1 h before the scopolamine administration. (F) Piracetam (100 mg/kg) treated group 1 h before the scopolamine administration.

Discussion

The present study was aimed to investigate the anti-amnesic effects of methanolic extract of *P. marsupium*. To assess efficacy, scopolamine was used to induce memory impairment in mice, and impairment was gauged using elevated plus maze and the Morris

water maze test. The neurotoxicity study indicated that the mice treated with higher dose of the *P. marsupium* (50 mg/kg) could not maintain equilibrium on the rotating rod suggesting neurological deficit. Whereas mice treated with lower dose of methanolic extract (25 mg/kg) were able to maintain equilibrium on the rotating rod for more than 5 min. So lower dose of the *P. marsupium* (25 mg/kg) was selected as most appropriate dose for further study.

Elevated plus maze was used to measure the anxiety state in animals, however transfer latency was markedly shortened if the animal had previous experience in entering open and closed arms, and this shortened transfer latency has been shown to be related with memory processes. Recent studies of several nootropics and amnesic agents on elevated plus maze made this model a widely accepted paradigm to study learning and memory processes in rodents. In elevated plus maze, acquisition (learning) can be considered as transfer latency on first day trials and the retention-n/consolidation (memory) is examined 24 h later. In our study, pretreatment with *P. marsupium* and piracetam for 7 days protected the animals from learning and memory impairment produced by interoceptive stimuli (scopolamine). The finding suggested the possible neuroprotective role for *P. marsupium* whereas, increase in IR (inflexion ratio) after 24 h indicated improved retention of learned task.

In another study, the methanolic extract of *P. marsupium* was evaluated to demonstrate its cognitive enhancing effects on spatial memory and learning function of mice against scopolamine-induced amnesic defects using Morris water maze test. In this test, methanolic extract of *P. marsupium* and piracetam treatment on the scopolamine-induced amnesic mice exhibited significant shorter escape latencies in daily 1st trial than the scopolamine administered groups during a 4-consecutive day training periods (Fig 1C-D), which suggest that extract improved the impaired reference memory (long term memory) induced by scopolamine. Further more the formation of working memory (short term memory) was revealed by significant differences in escape latencies between 1st and 2nd trial on day 1 in treatment groups (Fig 1 C-D). The results demonstrate that methanolic extract of *P. marsupium* improves spatial learning and memory function against scopolamine-induced amnesia.

The phytochemical tests of methanolic extract of *P. marsupium* showed the presence of various phytoconstituents viz. carbohydrates, glycosides, saponins, tannins and flavonoids. It is known that saponins compound have nootropic activities (Chintawar et al., 2002) which partially explain the mechanism of action of extract. Further studies are warranted to isolate the nootropic compound and to elucidate their mechanism of action. The task has been used extensively to study the neurobiological mechanisms that underlie spatial learning and memory, age-associated changes in spatial navigation and the ability of nootropic agents to influence specific cognitive processes. The neurochemical basis of learning and memory remains poorly understood, despite extensive experimental and clinical study. Although the role of central cholinergic system is fairly well established, the role of other neurotransmitter system can not be ignored (Hollendar et al., 1986). Since scopolamine induced amnesia was reversed by *P. marsupium*, it is possible that the beneficial effect on learning and memory was due to facilitation of cholinergic transmission in mouse brain. However further studies are required to identify the exact mechanism of action. The present findings conclude that methanolic extract of *P. marsupium* has potent nootropic activity. It is possible that the beneficial effect on learning and memory was due to facilitation of cholinergic-transmission

in mice brain. However, further studies are necessitated to identify the exact mechanism of action.

Acknowledgements

Authors would like to thanks Prof. (Dr) Ranjit Singh, Director, School of Pharmaceutical Sciences, Shobhit University for their technical assistance.

References

- Acharya KP, Rokaya MB (2005). Ethno botanical survey of medicinal plants traded in the streets of Kathmandu valley. *Scientific World* 3, 44-48.
- Bejar C, Wang RH, Weinstock M (1999). Effect of rivastigmine on scopolamine-induced memory impairment in rats. *European Journal of Pharmacology* 383, 231-240.
- Chintawar SD, Somani, RS, Veena S, Kasture SB (2002). Nootropic activity of *Albizia lebbek* in mice. *Journal of Ethnopharmacology* 81, 299-305.
- Dunham NW, Miya TS (1957). A note on a simple apparatus for detecting neurological deficit in rat and mice. *Journal of American Pharmaceutical Association Science* 46, 208-209.
- Frick K.M, Baxter MG, Markowska AJ, Olton DS, Price DL (1995). Age-related spatial reference and working memory deficits assessed in the water maze. *Neurobiology of Aging* 16, 149-160.
- Giurgea C (1973). The nootropic approach to the pharmacology of the integrative action of the brain. *Conditioned Reflex* 8, 108-115.
- Gordon I, Grauer E, Genis I, Sehayek E, Michaelson DM (1995). Memory deficits and cholinergic impairments in apo lipoprotein E-deficient mice. *Neuroscience Letter* 199, 1-4.
- Grover RK, Roy R, Maurya R (2004). Dynamic NMR investigation of two new interconvertible diastereomeric epimer of natural 2-benzyl-2-hydroxybenzofurone derivative from *Pterocarpus marsupium*. *Tetrahedron* 60, 2005-2010.
- Higashida A, Ogawa N (1987). Differences in the acquisition process and the effect of scopolamine on radial maze performance in the strains of rats. *Pharmacology Biochemistry and Behavior* 27, 483-489.
- Hollander E, Mohs RC, David KS (1986). Cholinergic approaches to treat Alzheimer's disease. *British Medical Bulletin* 42, 97-100.
- Jain A, Katewa SS, Galav PK, Sharma P (2004). Medicinal plants diversity of sitamata wild-life sanctuary, Rajasthan. *Journal of Ethnopharmacology* 102, 143-157.
- Jaiswal AK, Bhattacharya SK (1992). Effects of Shilajit on memory, anxiety and brain monoamines in rats. *Indian Journal of Pharmacology* 24, 12-17.
- Joshi MC, Dorababu M, Prabha T, Kumar MM, Goal RK (2004). Effects of *Pterocarpus marsupium* on NIDDM-induced rat gastric ulceration and mucosal offensive and defensive factors. *Indian Journal of Pharmacology* 36, 296-302.
- Khandelwal KR (2004). Practical Pharmacognosy (11th edn). Nirali Prakashan, Pune. pp. 149-156.
- Mankani KL, Krishna V, Manjunatha BK, Vidya SM, Singh SDJ, Manohara YN (2005). Evaluation of hepatoprotective activity of stem bark of *Pterocarpus marsupium* Roxb. *Indian Journal of Pharmacology* 37, 165-168.

- Morris RG (1984). Development of a water maze procedure for studying spatial learning in the rat. *Journal of Neuroscience Methods* 11, 47–60.
- Nabeshima T (1993). Behavioral aspects of cholinergic transmission: role of basal forebrain cholinergic system in learning and memory. *Progress in Brain Research* 98, 405–411.
- Nadkarni AK (1976). In Nadkarni's Indian Materia (vol. I). Popular Prakashan, Bombay. pp. 662-663.
- Rout SD, Panda T, Mishra N (2009). Ethnomedicinal plants used to cure different diseases by tribals of Mayurbhanj district of north Orissa. *Ethnomedicine* 3, 27-32.
- Sitaram N, Weingartner H, Gillin JC (1978). Human serial learning. Enhancement with arecholine and choline and impairment with scopolamine. *Science* 201, 247–276.
- Vats V, Grover JK, Rathi SS (2002). Evaluation of anti-hyperglycemic and hypoglycemic effect of *Trigonella foenum-graecum* Linn, *Ocimum sanctum* Linn and *Pterocarpus marsupium* Linn in normal and alloxanized diabetic rats. *Journal of Ethnopharmacology* 79, 95-100.
- Zimmermann M (1983). Ethical guidelines for investigation of experimental pain in conscious animals 16, 109-110.