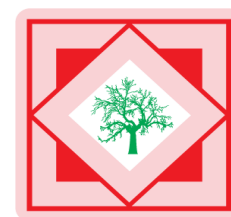




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Nootropic potential of *Murraya Koengii* leaves by Object Recognition test in rats

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ABSTRACT

The study was designed to evaluate the nootropic potential of *murraya koengii* leaves by using object recognition test in albino rats. The apparatus is composed of open box (16x5cm) with an open roof. The object recognition test includes 3 sessions A) The first day training session which consist of placing all the rats one by one in an empty recognition chamber for 5 minutes so that the rats get habituated in the environment (habituation). B) On Second day, test session begins (acquisition) where in the control rats are allowed to explore 2 different objects of same size, color and weight but with different shapes for period of 5 minutes (F&F1) and the time taken by each rat to explore the objects (rat touches its nose or places its nose at a distance of 2 cms from the object) was recorded. Repeat the same procedure with test and standard treated rats after one hour of administration of *murraya koengii* chloroform extract 100 mg/kg and Piracetam 200 mg/kg. C) The Third day session includes administration of an amnesic drug propofol (0.5 ml/200g) i.p to all the groups and then exploring the control, test and standard treated rats to one familiar object (F) and a new object (N) for a period of 5 minutes. Discrimination index which is an index of memory is calculated in all the groups of rats. It was found that discrimination index of *murraya koengii* chloroform extract 100 mg/kg treated rats was 0.714 ± 0.615 which is greater than the discrimination index of control treated rats (0.302 ± 0.014). This increase in discrimination index with *murraya koengii* leaf extract rats when compared to control rats indicates the presence of nootropic activity in *murraya koengii* leaves. Standard (piracetam 200mg/kg) treated rats exhibited discrimination index of 0.729 ± 0.192 . Phytochemical screening of *murraya koengii* leaves reveals the presence of carbohydrates, alkaloids, glycosides, flavonoids, proteins, triterpenes, resins and phytosterols. Hence any of the above mentioned active constituents in *murraya koengii* leaves may be responsible for its nootropic potential.

Key words: Nootropic activity, *Murraya koenigii*, discrimination index, object recognition test.

INTRODUCTION

In psychology, memory is the process in which information is encoded, stored and retrieved [1]. Cognition is the mental ability of developing knowledge and understanding through thought, experience and the senses [2]. Poor memory, slow recall and lower retention are common problems in today's world [3]. Memory declines mostly under stress and fatigue [4]. Several memory enhancing drugs are available in the modern medicine but with potentially toxic adverse effects [5]. The *Murraya koenigii* plant is widely used as herb, spice, condiments and also used to treat various types of ailments in Indian traditional system. World's about 80% population relies upon herbal products, because they have been considered as safe, effective and economical. The various parts of this plant are widely used by different tribal communities. The leaves of plant are use as tonic, stomachic, carminative, internally in dysentery,

vomiting [6, 7]. Used as antihelminthic, analgesic, cures piles, allays heat of the body, thirst, inflammation and itching [8, 9]. The present study was designed to investigate the memory enhancing activity of *Murraya koenigii* leaves by using object recognition test in rats.

MATERIALS AND METHODS

For the present study, the leaves of *Murraya koenigii* were collected from the surrounding gardens of the vanasthalipuram, hayathnagar (mandal), ranga redy dist, telangana, india. After the fresh leaves were authenticated by Dr. N. Sivaraj, Senior Scientist (Eco Botany), National Bureau of Plant Genetic Resources, Rajendranagar, Hyderabad, leaf specimens have been deposited at the museum of the college. Fresh mature leaves were shade dried at room temperature, coarse powdered with electric grinder and further extracted with chloroform by maceration for a period of 5 days. Thereafter, the extract was concentrated by evaporation. The percentage yield of the leaf extract was found to be 12.7%. The extract was stored in airtight container in refrigerator below 10°C. Appropriate concentration of stock solution of extract were prepared using span 80 and used for the following studies.

Preliminary phytochemical screening [10]

Preliminary phytochemical tests were performed for the chloroform extract of *Murraya koenigii* leaves to detect the presence of phytochemicals by following the standard methods described in the practical pharmacognosy of kokate and khandelwal. The results have been tabulated in table I.

Experimental animals

Albino rats (180-225g) were used in the present research. They were procured from sainath agencies, musheerabad (282/99/CPCSEA). After randomization into various groups and before initiation of experiment, the rats were acclimatized for a period of 10 days. Animals were housed in polypropylene cages and maintained under standard environmental conditions such as temperature ($26 \pm 2^\circ\text{C}$), relative humidity (45-55%) and 12hr dark/light cycle. The animals were fed with rodent pellet diet (Golden Mohur Lipton India Ltd.) and water *ad libitum*. The study protocol was approved from the institutional animal ethics committee (IAEC) before commencement of experiment IAEC (1292/ac/09/CPCSEA).

Determination of acute toxicity (OECD guidelines 423) [11]

The *Murraya koenigii* chloroform extract was studied for acute toxicity study at a dose of 100 mg/kg, 200mg/kg, 400 mg/kg, 800 mg/kg and 2000 mg/kg p.o in albino rats (6 rats in each group). All the rats were observed for toxicity signs for 24hrs followed by 14 days. On 8th day & 14th day body weight of rats were recorded. The rats were found toxic at the dose of 2000mg/kg i.e., rats exhibited signs of convulsions, drowsiness & ataxia. Hence 1000 mg/kg was selected as safe dose and 1/10 of 1000mg/kg i.e., 100mg/kg of *Murraya koenigii* leaves was selected for the studies.

Effect of Chloroform extract of *Murraya koenigii* leaves on memory by Object recognition test in rats:

The experiment was performed on albino rats (150-250gms) of either sex procured from sainath agencies, musheerabad. The animals were housed in colony cages at an ambient temperature of $26 \pm 2^\circ\text{C}$ and, relative humidity (45-55%) with a 12h/12h light dark cycle and access to food and water *ad libitum*. Food was restricted during experiments. Stock solutions of piracetam (200mg/kg) and Chloroform extract of *Murraya koenigii* leaves (100mg/kg) were prepared in 2% acacia suspension. Divide the animals into three groups control(C), test (T), and standard(S) each consisting of three rats. All the animals were subjected to object recognition apparatus assessment of nootropic levels. The apparatus is composed of open box (16x5cms) with an open roof. Two similar objects of same size, color, weight but with different shapes were placed at opposite corners in the box. The object recognition test includes 3 trials: The first day training session which consists of placing all the control rats one by one in an empty recognition chamber for 5 minutes so that the rats get habituated in the environment (habituation). After training trail, second day test session begins (acquisition), where in the control rats are allowed to explore 2 different objects (F&F1) of same size, weight and color but with different shapes for period of 5 minutes & the time taken by rat to explore the objects (rat touches its nose or places its nose at a distance of 2 cms from the object) was recorded. Repeat the same procedure with standard (piracetam 200 mg/kg) and test extract (*Murraya koenigii* 100 mg/kg) treated rats after 1 hr of administration of standard and test extract. The 3rd day session includes administration of propofol (amnesia inducer) to control rats and exploring the control rats to one familiar object (F) and a new object (N) for a period of 5 minutes. Repeat the same propofol treatment for test and standard treated rats after 1 hr of

administration of extract (*Murraya koenigii* 100mg/kg) and piracetam (200mg/kg) and the same exploration procedure was repeated (N&F) for test and standard treated rats.

Discrimination index was calculated as: $N-F/N+F$



Figure I: A) Object Recognition Test apparatus B) Objects used in Object Recognition Test

STATISTICAL ANALYSIS

The values are represented as mean $\pm$ S.E.M and statistical significance between treated and control groups was analyzed using One way ANOVA, followed by Dunnett's test where $P < 0.001$, $P < 0.01$ and $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Preliminary Phytochemical screening of chloroform extract of *Murraya koenigii* 100mg/kg leaves reveals the presence of flavonoids, triterpenoids, saponins, traces of tannins, carbohydrates, steroids, glycosides, alkaloids, proteins, resins, phytosterols, gum & mucilages. It is evident from the literature that flavonoids have potent memory enhancing ability. [12] Object recognition test is the most widely used *invivo* model for the assessment of memory. In object recognition test discrimination index is used as a factor for the assessment of memory. Discrimination index is determined in all the groups of rats. It was found that discrimination index of *murraya koengii* chloroform extract 100 mg/kg treated rats was 0.714 ± 0.615 which is greater than the discrimination index of control treated rats (0.3023 ± 0.014). This increase in discrimination index with *murraya koengii* leaf extract rats when compared to control rats suggests the presence of nootropic potential in *murraya koengii* leaves. Standard (piracetam 200mg/kg) treated rats exhibited discrimination index of 0.729 ± 0.192 indicating higher nootropic potential than test *murraya koengii* leaf extract.

Table I: Preliminary Phytochemical screening of chloroform extract of *Murraya koenigii* leaves

Phytoconstituents	chloroform leaf extract
Carbohydrates	+
Steroids	-
Glycosides	+
Flavonoids	+
Alkaloids	+
Tannins	-
proteins	+
Triterpenoids	+
Resins	+
Phytosterols	+
Gum & mucilages	-

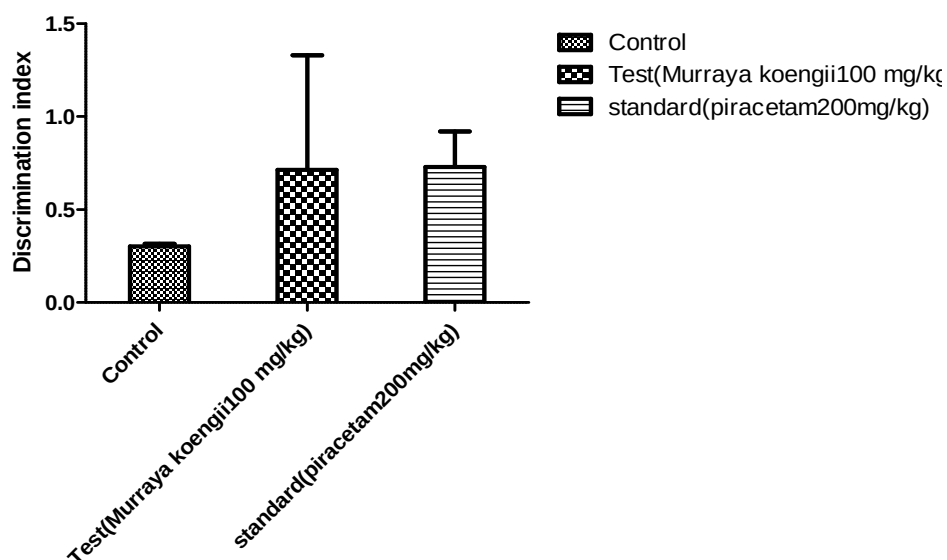
- Absent
+ Present

Table II: Discrimination Index with Control, Test and Standard treated rats

Groups	Discrimination Index
CONTROL	0.302 ± 0.014
TEST (<i>Murraya koenigii</i> leaves 100 mg/kg, p.o)	0.714 ± 0.615**
STANDARD (Piracetam 200 mg/kg, p.o)	0.729 ± 0.192***

$P < 0.001$ ***, $P < 0.01$ ** and $P < 0.05$ * was considered statistically significant.

Figure II: Discrimination index with control, test and standard treated rats



CONCLUSION

The data obtained from the present nootropic study by using object recognition model in rats indicates that the discrimination index with *Murraya koenigii* leaves at 100 mg/kg was greater than the discrimination index of control rats. As the discrimination index is an index of memory, increase in discrimination index with test extract indicates the nootropic potential of *murraya koenigii* leaves. It may be hypothesized that *murraya koenigii* leaves may have a neuroprotective mechanism on brain cholinergic neurons. However further phytochemical isolation of active constituents has to be done to know the exact mechanism involved in nootropic activity of *murraya koenigii* leaves.

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