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A STUDY ON PROTECTIVE ROLE OF PRUNUS AMYGDALUS ON ALCOHOL INDUCED RETROGRADE AMNESIA

Komati. Anusha[*1], Preethi Chavan[1], Jyothi Vadthya[1], G. Bhavana[1], Jabbarlal Naik[2] Korra, Suresh Naik[2]
[1]Teegala Krishna Reddy College Of Pharmacy, Medbowli, Meerpet, Saroornagar, Hyderabad, telangana JNTUH, 500097.
[2]Gurram Balanarsaiah College of Pharmacy, Edulabad, Ranga Reddy, Ghatkesar, Telangana JNTUH 501301.

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ABSTRACT

Ethanol and Prunus amygdalus (PA) are most widely used in the world. PA is a popular nourishing food, along with its nutritional values it also possess medicinal values like anti-stress, anti oxidant, immunostimulant, lipid lowering and laxative. Ethanol- induced retrograde amnesia was investigated using Morries Water Maze, Elevated plus Maze. Anxiety and locomotion was assessed using open field test. Rats received Prunus amygdalus suspension (150mg/kg, 300mg/kg, and 600mg/kg., oral) for 7 days, and Alcohol (3g/kg, 20%, i.p) in 6th day of the treatment. The next day they were accessible for Behavioral assessment. Rats receiving Alcohol shows significant reduction in escape latency (MWM), Time spent in open arm (EPM), Number of ambulations and rearing (OFT) and increase in Immobility time (FST). The current study evidenced learning and memory impairment, anxiety, and depression symptoms in rats characterized by behavioral impairment, increased oxidative stress, apoptosis, following exposure to ethanol. PA suspension helps in recovery of disrupted memory after ethanol inducing alterations in brain, ultimately, improved the memory (spatial and learning), anxiety and depression.

Corresponding author
Komati. Anusha
Assistant professor,
Department of Pharmacology,
Teegala Krishna Reddy College Of Pharmacy,
Medbowli, Meerpet, Saroornagar, Hyderabad, JNTUH, 500097.
anusha.komati77@gmail.com
9849640482

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INTRODUCTION

Cognitive disorders such as amnesia, attention deficit and Alzheimer's disease are emerging nightmares in the field of medicine because no exact cure exists for them, as existing nootropic agents (piractam, tacrine, metrifonate) have several limitations. The present study was undertaken to investigate the effect of *Prunus amygdalus* (PA) nuts on cognitive functions, anxiety and depression in ethanol-induced retrograde amnesia in rats**.** Amnesia is divided into retrograde amnesia and anterograde amnesia. Retrograde amnesia refers to the ability of a patient to lose the ability to recall the event memory before a brain injury.

These days we find people using alcoholic beverages during different occasions which cause effects on CNS. High doses of ethanol can cause amnesia, or impaired retrieval of memory, evidenced after the drug wears off (Goodwin 1995; Hartzler and Fromme 2003; Wixted 2005). Consuming greater amounts of alcohol may contribute to harsher, more acute depressive symptoms [1].

mnesia has several origin causes. Most are perceptible to brain injury related to physical trauma, disease, infection, drug and alcohol abuse, or abridged blood flow to the brain (vascular insufficiency). Alcohol treatment to rats which readily crosses the blood–brain barrier and is metabolized in the brain which produces reactive oxygen species (ROS) riot of cellular normal redox state by excessive ROS leads to oxidative stress which causes cellular damage [2] and induces retrograde amnesia [3].

Prunus amygdalus (Rosaceae; PA) is a small tree indigenous to regions around the Mediterranean Sea. Almonds or badam, and is a popular nourishing food [4] Almonds are rich in healthy fats, proteins, minerals and vitamins. In addition to its nutritional values, it has some medicinal values that may be helpful for treating certain diseases and health problems. The almond is an effectual health edifice food, both for the body and the mind; it is also a precious food remedy for several common ailments. The nuts of Prunus amygdalus are have various pharmacological properties, such as anti-stress [5], anti-oxidant [6], immunostimulant [7], lipid lowering [8] and laxative [9].

The almond is highly valuable in preserving the vitality of the brain, reinforcement the muscles and prolonging life. Various behavioral responses produced by Prunus Amygdalus therapy on exposure of alcohol in rats were assessed by using tests like morries water maze, elevated plus maze, forced swim test, open field test. Prunus amygdalus attenuated exposure of alcohol induced alterations in brain, eventually, improved the memory.

MATERIALS AND METHODS

Animals

Male Wister rats weighing between 180-200 g were obtained. Rats were housed 6 per cage, allowed access to water and food ad libitum, and maintained in constant temperature (23 ± 2 °C) and humidity (55 ± 5%) under a 12 h light/dark cycle. The rats were allowed free access to food (Standard pallet) and water. This study protocol was approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) code: NCOP/3166/2013

Drugs and chemicals

The nuts of PA were purchased from the local market and authenticated at the botany department of Agharkar Research Institute, Pune, India. Ethanol has purchased from premier ethanol Pvt. LMT, Hyderabad, India.

Method of preparation and Dose administration

The fine paste of the PA nuts was prepared by using distilled water and fine suspension was obtained by using sonicator for 20 min. Then, the three doses of 150, 300 and 600 mg/kg/day paste was administered to rats. The above dose levels were selected by the conversion of conventional human dose into animal dose. The human dose of PA is five to six nuts daily (approximately 6 g). PA was administered at the same time on each day (i.e., 8.00-9.00am) for 7 days. Ethanol was administered (3g/kg, 20%, i.p) on 6^{th} day of the treatment.

Experimental design

Total 10 animals were randomly divided into five different groups, each group containing 6 rats.

I) Control Group – Receives distilled water.

II) Alcohol Group – Receives Alcohol (3g/kg, 20%, i.p) on 6^{th} day of the treatment

III) Alcohol+ Dose-I Group – Receives Alcohol (3g/kg, 20%, i.p) on 6^{th} day of the treatment+Prunus amygdalus (150mg/kg/day, oral) for 7 days

IV) Alcohol+ Dose-II Group – Receives Alcohol(3g/kg, 20%, i.p) on 6^{th} day of the treatment+ Prunus amygdalus (300mg/kg/day, oral) for 7 days

V) Alcohol+ Dose-III Group – Receives Alcohol(3g/kg, 20%, i.p) on 6^{th} day of the treatment+ Prunus amygdalus(600mg/kg/day, oral) for 7 days.

BEHAVIORAL STUDY

Morris Water Maze Test

Morris water maze test is used to test to include acquisition of spatial memory. The method described by Saraf et al. (2011) and Dhingra and Kumar (2012) was adopted for the present study. Morris water maze (142 × 60 cm) consisted of a circular pool (28 × 13 cm) filled up to a depth of 14 cm with water maintained at 25□C. Water was made opaque by adding titanium dioxide. The tank was divided into four equal quadrants by using two threads fixed at right angles to each other on the rim of the pool. A submerged platform was placed inside the tank at the centre of the pool 1 cm below water level. The position of the plat form was unaltered throughout the training session. Each animal was subjected to four consecutive trials each day with different points with 5 min gaps between each trial for four consecutive days, during which they are allowed to escape on the hidden platform and to remain there for 20 sec. During the training session the rat was gently placed in the water from different locations facing the wall of the pool and allowed 120 sec to locate the submerged platform. If the rat failed to locate the platform within 120 sec, it was guided gently on to the platform and allowed to remain there for 20 sec. Each animal was subjected to training trials for four consecutive days and the starting position was changed with each exposure as mentioned below:

Day 1: (A), (B), (C), (D)
Day 2: (B), (D), (A), (C)
Day 3: (C), (B), (A), (D)
Day 4: (D), (C), (A), (B)

On the last day (retrieval day) of training session (i.e. Day 5), the platform was removed and the rat was placed in the pool from any of the point and allowed to explore the target quadrant (centre of the pool where the platform was placed) for 300 sec. The mean time spend in the centre of the pool in search of the missing platform (i.e. time spend in target quadrant, TSTQ), which is an index of retrieval or memory, was recorded [10].

Elevated Plus Maze

Transfer latency (TL) of each animal was measured by employing the elevated plus maze test. The plus-maze consisted of two open (16 x 5 cm) and two closed (16 x 5 x 12 cm) arms, connected by a central platform of 5 x 5 cm. The apparatus was elevated to a height of 25 cm above the floor. A fine line was drawn in the middle of the floor of each closed arm. All the animals were then given a single trial on the plus-maze. Each mouse was individually placed at the end of an open arm facing away from the central platform of the maze. TL was then taken as the time taken by the mouse to move from an open arm to any one of the closed arms with all its four legs crossing the middle line. In case, the animal did not enter the closed arm within 90 seconds it was gently pushed into the closed arm and a transfer latency of 90 seconds was assigned to it. After an interval of 24 hours each animal was again subjected to elevated plus-maze test. TL measured on plus-maze on day 7 served as an index of learning and acquisition, whereas TL on day 8 serves as an index of retrieval and memory [34-35]. (Fig 2) Fig 2: Diagrammatic representation of experimental protocol for drug administration and behavioral study (elevated plus maze) [11].

Forced Swimming Test (FST)

Forced swimming test, a representative behavioral testfor depression, is frequently used to evaluate the activities of potential antidepressant drugs in rodent models. Forced immersion of rats in water for an extended period produces characteristic behaviors of immobility. The antidepressant treatments decrease the immobility behavior accompanying with an increase in the escape responses such as climbing and swimming behaviors. A transparent Plexiglas cylinder (20 cm diameter×50 cm height) was filled up to a depth of 30 cm with water at 25 ^{o}C. At this depth, rats could not touch the bottom of the cylinder with their tails or hind limbs. On day 14, the rats in all groups were trained for 5 min by placing them in the water-filled cylinder. On day 15, animals were subjected to 5 min of forced swim, and escape behaviors (climbing and swimming behaviors) were determined. The duration of immobility was scored during the 5 min test period. Climbing behavior was defined as upward-directed movements of the forepaws alone the side of the swim chamber and swimming behavior was considered as movements throughout the swim chamber including crossing into another quadrant. Immobility behavior was calculated as the length of time in which the animal did not show escape responses (e.g., total time of the test minus time spent in climbing and swimming behaviors). The animals' behavior was continuously recorded throughout the testing session with an overhead video camera. After the test, the rat was removed from the tank, dried with a towel and placed back in its home cage. The water in the swim tank was changed between rats [12, 13].

Open Field Test

Prior to forced swimming test, the rats were individually housed in a rectangular container that was made of dark polyethylene (60×60×30 cm) to provide best contrast to the white rats in a dimly lit room equipped with a video camera above the center of the room, and their locomotor activities (animal's movements) were then measured. The locomotor activity indicated by the speed and the distance of movements was monitored by a computerized video- tracking system using S-MART program (Panlab Co., Barcelona, Spain). After 5 min adaptation, the distance they traveled in the container was recorded for another 5 min. The locomotor activity was measured in centimeters. The floor surface of each chamber was thoroughly cleaned with 70% ethanol between tests [14].

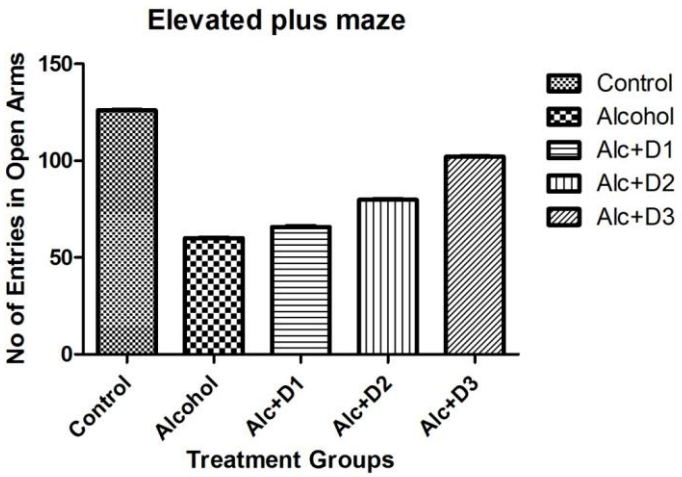
STATISTICAL ANALYSIS

Data are represented as mean±SEM (n=6). Results for all parameters were analyzed using one way ANOVA followed by post hoc the Dennett test control. The level of significance was set at $p \leq 0.05$. The analyses were performed with the Graph-Pad Instats5.0 and Graph-Pad Prism statistical package 5.0.

RESULTS

Behavioural Parameters

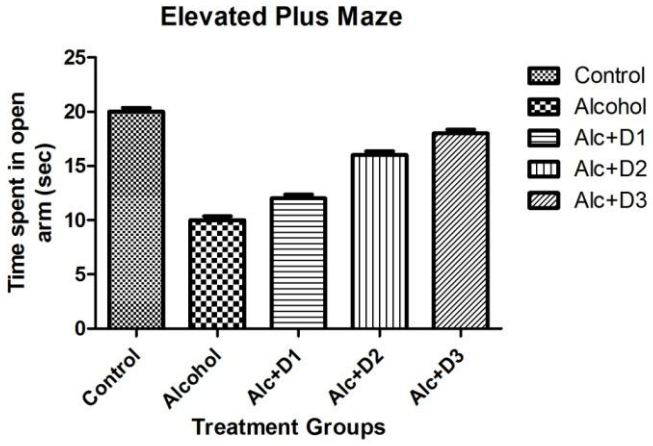
Number of entries in open arm.



Values are expressed as Mean±SEM, n=6. *P<0.05 compared with control, #P<0.05 compared with alcohol.

Figure1. Effect of Prunus amygdalus on *Elevated plus maze activity* in learning and memory impairment and anxiety induced rats

Time spent in open arm (sec).

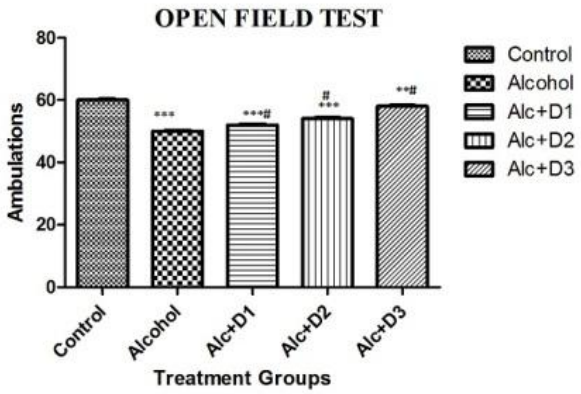


Values are expressed as Mean±SEM, n=6. *P<0.05 compared with control, #P<0.05 compared with alcohol.

Figure2. Effect of Prunus amygdalus on *Elevated plus maze activity* in learning and memory impairment and anxiety induced rats.

Anxiety behaviour was assessed using elevated plus maze performance. Administration of alcohol showed a significant decrease ($p<0.05$) in number of entries into open arm and time spent in open arm compared to control, (Figure.1 & 2). Treatment with Prunus amygdalus significantly ($p<0.05$) increase in number of entries in to open arm and time spent in open arm compared to alcohol exposed animals.

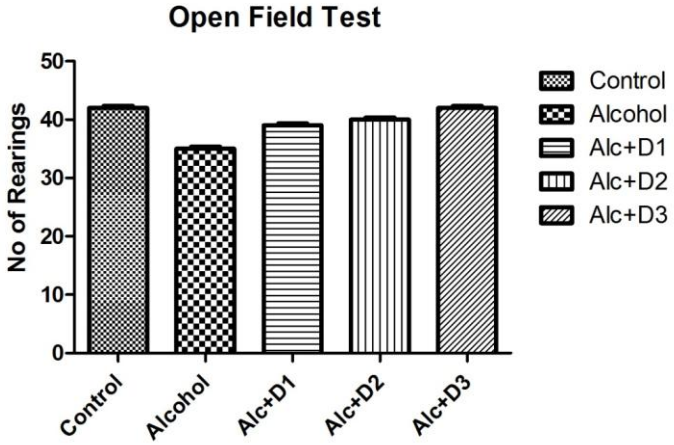
Ambulations.



Values are expressed as Mean±SEM, n=6. *P<0.05 compared with control, #P<0.05 compared with alcohol.

Figure3. Effect of Prunus amygdalus on *Open field activity* in learning and memory impairment and anxiety induced rats.

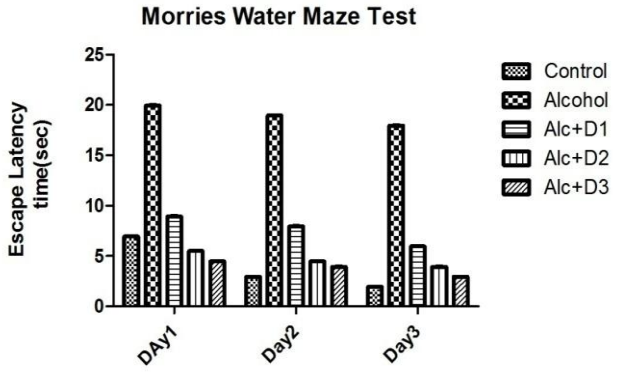
Rearings:



Values are expressed as Mean±SEM, n=6. *P<0.05 compared with control, #P<0.05 compared with alcohol.

Figure4. Effect of Prunus amygdalus on *Open field activity* in learning and memory impairment and anxiety induced rats.

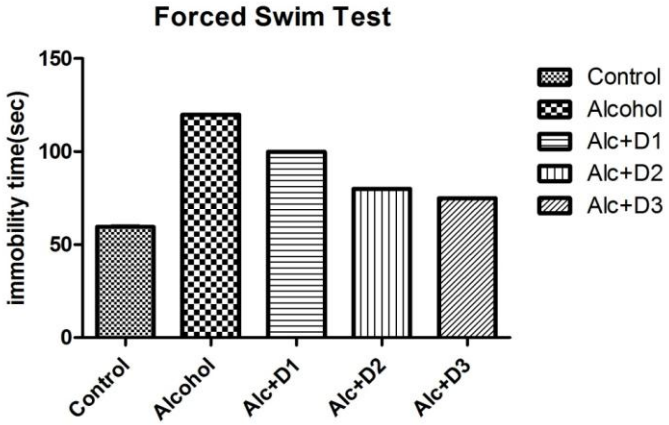
Locomotor activity was assessed by using open field. Administration of alcohol, showed a significant (p<0.05) reduction in number of ambulations, number of rearings compared to control, (Figure.3 & 4). Therapy with Prunus amygdalus significantly (p<0.05) increased the number of rearings and number of ambulations compared to alcohol exposed animals.



Values are expressed as Mean±SEM, n=6. *P<0.05 compared with control, #P<0.05 compared with alcohol.

Figure5. Effect of Prunus amygdalus on *Morries water maze activity* in learning and memory impairment and anxiety induced rats.

Spatial learning and memory were assessed using water maze. Alcohol administered animals showed significant (p<0.05) alteration in the escape latency compared to control animals, same (Figure.5). Therapy of Prunus amygdalus significantly (p<0.05) attenuated impairment in spatial learning and memory induced by alcohol. Compared to Day1results, Day2 and Day3results are not significantly (p<0.05) increased.



Values are expressed as Mean±SEM, n=6. *P<0.05 compared with control, #P<0.05 compared with alcohol.

Figure6. Effect of Prunus amygdalus on *Forced swim test activity* in learning and memory impairment and anxiety induced rats.

Forced swimming test is a representative behavioral test for depression. Alcohol administered animals showed significant (p<0.05) increase in the immobility time compared to control animals, same (Figure.6). Therapy of Prunus amygdalus not significantly (p<0.05) attenuated impairment in immobility time induced by alcohol.

DISCUSSION

Retrograde amnesia mainly occur due to brain injury in areas such as medial, temporal lobe, diencephalon and frontal lobe. Alcohol consumption, lacking of vitamin B1 and trauma may also leads to RA. Ethanol readily cross BBB and shows specific action in the hippocampus, particularly ethanol enhance GABAergic potency at GABA-A receptor by increasing levels of allopregnanolone, interfere glutamate activity at NMDA receptors it leads to spatial memory impairment and also reduce hippocampus acetylcholine levels. Ethanol also reduce the serotonin levels and other chemicals in the brain, may leads to the development of depression, anxiety.

PA possesses memory enhancing activity in view of its enhancing effect on the retention of spatial memory in alcohol induced retrograde amnesia. There is a decrease in the Transfer Latency, i.e. rats were able to recognize the dark zone immediately after exposure to the open arm in the Elevated plus Maze, which is an indicator of cognition improvement.

Open field are Forced Swim test paradigms employed to screen locomotory and depression respectively. Exposure to alcohol is believed to evoke anxiety and decrease locomotory exploratory behaviour exaggerated fear. Evaluation of the pattern of exploration in an open field following treatment with Prunus amygdalus suggests that stereotypic forms of locomotion were significantly controlled and exploratory activity was neutralized.

Therefore, the memory-improving activity of PrunusAmygdalus may be attributed to the anti-AChE, Procholinergic, cholesterol reduction, neuroprotective and nutritive properties of the PA nuts. Hence, PA may be used in delaying the onset and reducing the severity of Alzheimer's disease. However, further investigations are warranted to explore the possible involvement of other neurotransmitters such as glutamate, Gamma aminobutyric acid (GABA) and catecholamines, responsible for the memory-improving property of PA.

CONCLUSION

Based on the present study learning and memory impairment, anxiety, and depression symptoms in rats characterized by behavioral impairment, increased oxidative stress, apoptosis, due to exposure of alcohol, which progressively increased the oxidative damage in hippocampal neurons. Prunus amygdalus (150mg/kg, 300mg/kg, 600mg/kg) attenuated exposure to alcohol induced alterations in brain, ultimately, improved the memory (spatial and learning) of rats on administration for 7 days.

PA can be explored for pharmacological effectiveness in prevention and management of cognitive dysfunction.

List of abbreviations:

PA : Prunus amygdalus
RA : Retrograde amnesia
GABA : Gama amino butyric acid
NMDA : N- methyl D- aspartate
BBB : blood brain barrier

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Conflict of interest:

The authors declare that they have no conflicts of interest.

REFERENCES

1. NHS Choices, "Clinical Depression", NHS, (August 2014), [accessed 1/4/15] 3 L Knott, "Depression"[O]
2. Goldstein S, Merenyi G. The chemistry of peroxynitrite: implications for biological activity. Methods Enzymol. 2008; 436:49–61. [PubMed]
3. Berryman CE, Preston AG, Karmally W, Deckelbaum RJ, Kris-Either ton PM. et.al., Effects of almond consumption on the reduction of LDL-Cholesterol: a discussion of potential mechanisms and future research directions. J Nutr. Rev 2009; 69:171-85.
4. Tran TD, Jackson HD, Horn KH, Goodlett CR. Vitamin E does not protect against neonatal ethanol-induced cerebellar damage or de ficits in eyeblink classical conditioning in rats. Alcohol ClinExp Res 2005; 29:117 –29.
5. Roebuck TM, Mattson SN, Riley EP. A review of the neuroanatomical fi ndings in children with fetal alcohol syndrome or prenatal exposure to alcohol. Alcohol ClinExp Res 1998; 22:339 – 44.
6. Crews FT, Nixon K. Mechanisms of neurodegeneration and regeneration in alcoholism. Alcohol 2009; 44: 115 –27.
7. Haorah J, Ramirez SH, Floreani N, Gorantla S, Morsey B, Persidsky Y. Mechanism of alcohol-induced oxidative stress and neuronal injury. Free RadicBiol Med 2008; 45: 1542 –50.
8. Hampton M B, Orrenius S. Redox r egulation of apoptotic cell death. Biofac tors 19 98; 8: 1 – 5.
9. AnshulTanwar, Pallavi A Bafna, Anand R Bafna, Anti-amnesic effect of aqueous extract of Crataevanurvalastem bark in scopolamine induced amnesia, Journal of Applied Pharmaceutical Science Vol. 4 (09), pp. 066-072, September, 2014, Available online at http://www.japsonline.com , DOI: 10.7324/JAPS.2014.40912, ISSN 2231-3354., Department of Pharmacology, Rayat Institute of Pharmacy, Railmajra, Punjab, India.
10. Hebbar SS, Harsha VH, Shripathi V, Hegde GR. 2004. Ethnomedicine of Dharward district in Karnataka, India-plants used in oral health care. J Ethnopharmacol 2004; 94: 261-266.
11. Darks AM, Oestreicher AB, Gispen WH, Isaakson RL. Behaviour and anatomical sequences of unilateral fornix lesion and administration of nimodipine. Brain res.1991; 557: 308-312.

12. Furuzan YB, Faruk E, Güner TU, Nejat G. Antidepressant-like effect of 7-nitroindazole in the forced swimming test in rats. Psychopharmacology, 2000; 149:41 29.
13. Fujimori H, Cobb D. Central nervous system depressant activity of MA 1337, 3-[3,4-m-clorophenyl-I-piperazyl) propyl)-2-qlH,3H) quinozolinedione hydrochloride. The Journal of Pharmacology and Experimental Therapeutics 1965; 148: 151-157.
14. Cícero Francisco B. F, Sales K F, Barbosa1 ALDR., Bezerra JNS, Manoel A N, Marta Maria de FF, and Glauce SD BV. Alterations in behaviour and memory induced by the essential oil of Zingiber officinale Roscoe (ginger) in mice are cholinergic-dependent. Journal of Medicinal Plants Research 2008; 2(7): 163-170.

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