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ESTIMATION OF TOTAL FLAVONOIDAL CONTENT AND NOOTROPIC ACTIVITY OF *LEPIDIUM SATIVUM*

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Keywords:

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ABSTRACT: Objective: *Lepidium sativum* (Garden cress) is traditionally an important plant with a wide range of activities from the ages. Ample literature about its biological activities, motivate us to evaluate the total flavonoids content and to screen the nootropic activity in mice. **Methods:** Ethanolic extract was prepared from the seeds of *Lepidium sativum* using ethanol (70% v/v) and total flavonoids content was estimated by using Zhishen method. The ethanolic extract was screened for its acute toxicity and nootropic activity by elevated plus maze (EPM) and morris water maze (MWM) test in mice. **Results:** The results showed that the ethanolic seed extract of the plant is having a good amount of flavonoids (17.54 ± 47 of gram equivalence of rutin at 510 nm) and also exhibited significant increase in memory and learning abilities. **Conclusion:** To conclude, the flavonoids that are present in the ethanolic extract may be responsible for the exhibited activity and it is regarded as safe to administer to the animals. Moreover, further studies are necessary to explore its mechanism of action.

INTRODUCTION: *Lepidium sativum*, commonly called as Asalika, is a small edible herb which can grow up to 15-50 cm height throughout the country. The seeds are reddish-brown in color with ovate shape having a slight compression and reticulation on the surface. The seed is rich with mucilaginous material with incumbent cotyledons [1]. The seeds are pungent and odorless. Traditionally it is used to cure various ailments like skin infections, pains, inflammations, liver problems, gastric disturbances, asthma, *etc.* It is also used as aphrodisiac, diuretic and tonic [2, 3].

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Various researchers worked on various extracts of the plant to prove huge pharmacological properties. It is having anti-diabetic property [4, 5], anti-diarrheal property [6], anti-oxidant activity [7], gonadotropin secretion property [8], anti-cholesterol property [9], hypothyroid property [10], anti-hepatitis activity [11], Immunostimulant property [12], hepatoprotective property [13, 14], fracture healing property, anti-hypertensive, diuretic, hepatoprotective, nephro-curative, nephroprotective activity, anticancer activity, bronchoprotective, galactagogue property, analgesic, anti-inflammatory, anti-pyretic and anti-microbial activity [15, 16, 17].

The phytochemical investigation on various extracts of *Lepidium sativum* seeds revealed various group of chemicals like monomeric imidazole semilepidinoside A and B, dimeric imidazole alkaloids like lepidine B, C, D, E and F, Sinapic acid, glucosinolates like glucotropaeolin 2-

Phenyl ethyl glucosinolate also called gluconasturin, sterols like campesterol, avenasterol, cholesterol, stigmasterol, dihydrolanosterol, and ß-amyrin, carotenoids [18], antioxidants like tocopherols, phenolic compounds like caffeic acid, gallic acid etc and flavonoids like kaempferol and quercetin [19], cardiac glycosides and anthraquinones glycosides [20, 21]. Flavonoids are phenolic compounds with the basic moiety of fifteen carbons as two phenyl rings and one pyran ring. Because of their unique structural diversity, flavonoids are playing a key role in various biological activities like binding to the biomolecules, chelating metal ions and as free radical scavengers [22].

They are having significant importance in the management of neurodegenerative disorders like Alzheimer's disease by elevating the Ach levels at cerebral cortex synapse. In addition, flavonoids are antioxidant in nature; they are effective in stress-induced neurodegenerative diseases [23]. In the search of drugs for enhancing cognitive abilities and memory our present investigation is aimed to prove the nootropic activity of total flavonoids content of *Lepidium sativum* seeds.

MATERIALS AND METHODS:

Plant Material: The *Lepidium sativum* seeds were procured from the local suppliers and authenticated by Dr. N. Sivaraj, Senior Scientist (Ecobotany), National Bureau of Plant Genetic Resources, Rajendra Nagar, Hyderabad and a sample voucher (AU/LS/S154) was preserved at the herbarium for future references.

Extraction Procedure: The plants were shade dried and powdered using a pulverizer. The powder was subjected to exhaustive extraction on soxhlet using ethanol (70% v/v). The resulted extract (LS) solution was evaporated to dryness using rotary evaporator under vacuum at 60 ºC and lyophilized to have it in a powdered form.

Estimation of Total Flavonoidal Content of the Extract: The flavonoids content of the ethanolic seed extract was determined by a colorimetric method using aluminum trichloride method (Zhishen method) [24]. A volume of 125 µL of ethanolic extract is added to 75 µL of a 5% sodium nitrite ($NaNO_2$) solution. After 6 min, 150 µL of $AlCl_3$ solution (10%) was added followed by the addition of 750 µL of NaOH (1M). The final volume of the solution was made to 2500 µL with distilled water. After 15 min of incubation, the mixture turned to pink and the absorbance was measured at 510 nm. The total flavonoids content was expressed as gram equivalence of rutin per gram dry weight.

Acute Toxicity Studies: Albino Mice with about 25-35 gm were selected for this experiment. Mice were supplied from Sainath Agencies, Musheerabad. Polypropylene cages were used for the maintenance of animals at animal house during the experiment. Animals were acclimatized to standard laboratory conditions for one week and freely allowed to feed on standard rodent pellet diet (Golden Mohur Lipton India Ltd.) and water *ad libitum*. The temperature (25 ± 2 ºC), relative humidity (60% ± 10%) and 12 h dark/light cycle were maintained throughout the experiment [25]. The study protocol was approved from the Institutional Animal Ethics Committee (IAEC) before the commencement of experimental studies (CPCSEA/ 1657/IHECCMRCP/22/65).

Elevated Plus Maze: The retention and memory can be assessed by elevated plus maze [26]. The maze is made up of two open arms (50 × 10 cm) located opposite to each other and crossed with another two closed arms with the same length and width with a connected central square (10 × 10 cm). The entire system was kept at 50 cm height from the ground. Swiss albino mice weighing about 30-35 gms were selected for the study and grouped into four with six animals in each. Group 1 was supplied with distilled water (Control), Group 2 received standard Piracetam (100 mg/kg, p.o.), Group 3 and Group 4 received 250 and 450 mg/kg body weight of ethanolic seed extract of *Lepidium sativum.*

On the first and second day, one animal at a time was placed on the edge of an open arm and time taken for (transfer latency) the mice to enter one of the closed arms was noted. The mice were allowed to stay in the open arm for 10-15 sec and returned to the cage. On the 7th day, after the regular treatment with the dose again the transfer latency (TL) was noted.

Morris Water Maze (MWM) Test: It is having a circular water pool (45 × 26 cm) with depth 20 cm and the water temperature should be maintained at

26 ± 1 ºC. The pool was labeled with four directions namely, N, E, S and W. Milk was added to the water to make the water non-transparent. An escape platform was arranged in the pool which is dipped 1 cm in the water in SW direction and kept unchanged during the experiment. The animals are allowed find the platform only through stable distal spatial cues arranged at the testing room. Four different starting points (N, E, SE, NW) were made in the pool and all these starting were randomly used during the experiment.

In the experiment, the animal must find the platform within 2 min after placing on the starting point, facing towards the wall of the pool. After reaching the platform, the animal is allowed for 30 seconds on the platform. Escape latency time (ELT) to locate the hidden platform in the water maze was noted as an index of learning [27].

Statistical Analysis: All the results were expressed as mean ± SEM. One-way ANOVA, followed by Dunnett's post hoc test using the software GraphPad Prism 5 (San Diego, CA, USA) was used to compare the groups, and P<0.05 was considered statistically significant.

RESULTS AND DISCUSSION:

Total Flavonoid Content of the Extract: The flavonoid content of the ethanolic seed extract was determined by a colorimetric method using Zhishen method, and it is found to be 17.54 ± 47 of gram equivalence of rutin at 510 nm.

Acute Toxicity Studies: Animals administered with acute doses of the extract did not develop any significant clinical signs of toxicity or mortality either immediately or during the post-treatment even at high dose of 3000 mg/kg body weight. The food intakes in both sexes of treatment groups were comparable to that of control group. These findings suggest the broad range of safety of *Lepidium sativum* **Table 1**.

TABLE 1: EFFECT OF *LEPIDIUM SATIVUM* ETHANOLIC SEED EXTRACT IN ALBINO MICE

Group	Dose in mg/kg	Bodyweight in grams			Outcome
		Day 0	Day 7	Day 14	
1	100	28	30	33	Survival (6/6)
2	250	27	29	32	Survival (6/6)
3	500	28	29	31	Survival (6/6)
4	1000	29	31	33	Survival (6/6)
5	1500	28	30	32	Survival (6/6)
6	2000	27	29	32	Survival (6/6)
7	3000	29	31	32	Survival (6/6)

Elevated Plus Maze: Pretreatment of Piracetam (200 mg/kg, i.p.) for 7 days decreased transfer latency as compared to distilled water treated group, indicating improvement in both learning and memory **Table 2**. LS (450 mg/kg) decreased TL on the 7th day in mice (p<0.05) when compared to respective control groups. Higher dose of LS (450 mg/kg) improved learning and memory of mice as reflected by marked decrease in TL on the 7th day, when subjected to elevated plus maze tests **Fig. 1**.

TABLE 2: EFFECT OF *LEPIDIUM SATIVUM* ETHANOLIC SEED EXTRACT ON TRANSFER LATENCY OF MICE USING ELEVATED PLUS-MAZE PARADIGM

Group	Treatment	TL after 7 days
1	Control	21.13 ± 0.65
2	Piracetam	12.75 ± 0.83
3	LS (250 mg/kg)	17.77 ± 0.45
4	LS (450 mg/kg)	15.25 ± 0.37

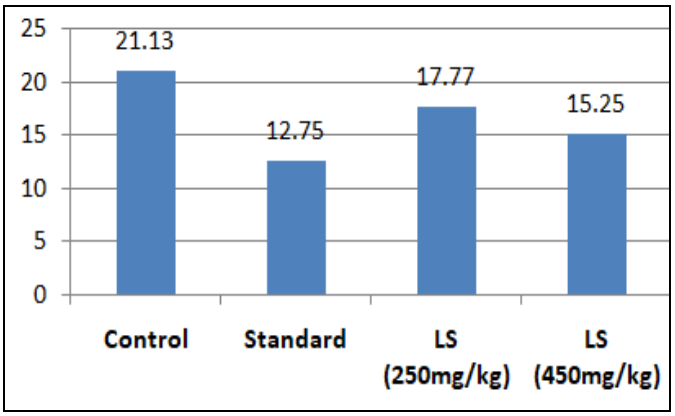

FIG. 1: EFFECT OF *LEPIDIUM SATIVUM* ETHANOLIC SEED EXTRACT ON TRANSFER LATENCY OF MICE USING ELEVATED PLUS-MAZE PARADIGM

Elevated plus-maze is extensively used for the evaluation of memory and learning process in experimental animals. The learning can be assessed based on the decreased latency time in the trials

and the retention of the memory can be determined on next day trials. In our experiment the ethanolic seed extract of *Lepidium sativum* was markedly reduced the transfer latency in the 7 days pretreatment which indicates the nootropic activity of the extract in mice.

Morris Water Maze (MWM) Test: The ethanolic seed extract of *Lepidium sativum* have marked learning capability in a dose-dependent manner in mice **Table 3**. When compared to the standard Piracetam (12.75 ± 0.83), the extracts had shown significant decrease in escape latency time in 250 and 450 mg/kg as 17.7 ± 0.45 and 15.25 ± 0.37 respectively after 7 days treatment of the extract **Fig. 2**.

TABLE 3: EFFECT OF *LEPIDIUM SATIVUM* ETHANOLIC SEED EXTRACT ON TRANSFER LATENCY OF MICE USING MORRIS WATER MAZE (MWM) TEST

Group	Treatment	TL after 7 days
1	Control	35.78±0.67
2	Piracetam	17.56±0.52
3	LS (250 mg/kg)	28.31±1.13
4	LS (450 mg/kg)	23.75±0.40

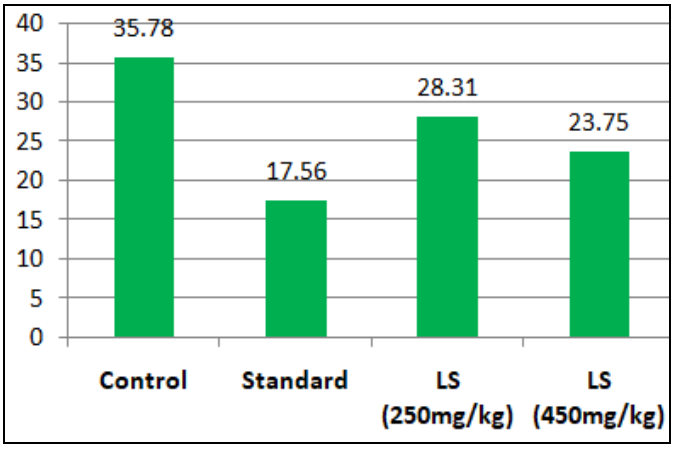

FIG. 2: EFFECT OF *LEPIDIUM SATIVUM* ETHANOLIC SEED EXTRACT ON TRANSFER LATENCY OF MICE USING MORRIS WATER MAZE (MWM) TEST

The learning function of the ethanolic seed extract of *Lepidium sativum* was also evaluated by Morris water maze test in mice. In this study, the extract decreased the escape latency time when compared to the control in a dose-dependent manner, which suggests the cognitive enhancing effect and working memory. There is a significant enhancement of the retention of the memory from the first trial to the last day trial, suggesting the improved long-term memory.

SUMMARY AND CONCLUSION: From the above study it can be concluded that the *Lepidium sativum* seeds are rich in flavonoids and the ethanolic seed extract is having the potential to improve memory and cognitive abilities in mice. Therefore, the seeds can be used to control the memory-related issues in neurodegenerative diseases like Alzheimer's diseases. Flavonoids present in the seeds may be responsible for the studied activity. Further investigation is needed in the phytochemistry and advanced experimental paradigms to understand the molecular mechanism of the nootropic activity of the seed.

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CONFLICTS OF INTEREST: Nil

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