ISSN: 0975-766X
CODEN: IJPTFI
Available Online through
www.ijptonline.com
Research Article

EFFECT OF ETHANOLIC EXTRACT OF *MORINGA OLEIFERA*LAM PODS ON LEARNING AND MEMORY IN STREPTOZOTOCIN INDUCED DIABETIC MICE

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Received on 25-02-2016 *Accepted on 22-03-2016*

Abstract

Aim:

The present investigation aims to investigate the effect of different doses of ethanolic extract of pods of *Moringa oleifera* as neurobeneficialin Diabetic stressed mice.

Material and Methods:

Swiss Albino mice of either Sex were administered STZ(75mg/kg) for Diabetes induction. Then mice were received injection of Ethanolic extract of *Moringa oleifera*(100, 200mg/kg) and the effects on learning & memory were studied.

The behaviors evaluated by using: Elevated plus Maze apparatus, Passive avoidance test, Object recognition test and locomotor activity were observed by using Actophotometer.

Statistical analysis: The results are expressed as mean + SEM**.** Comparison between the groups was made by one way analysis of variance (ANOVA) followed by Dunnett's test.

Results:

The acute administration of extract showed significant increase in exploration time to novel object in object recognition test, decrease in transfer latency and number of mistakes in passive avoidance and Elevated plus maze test, and decreased locomotor activity in Actophotometer.

Conclusion:

The result indicates the potential activity of the Moringa oleifera extract as a promising nootropic effect on STZ-induced diabetic mice.

Key wards: Nootropic, Moringa oleifera, Streptozotocin.

Introduction

The *Moringa oleifera*has been widely used in traditional medicine to treat variety of disease including some neurological disorder. Stress involves complex biochemical, neural and immunological mechanisms and plays a crucial role in the genesis and progression of variety of diseased states.

[1]As Stress is very predominant in this modern world, there is a definite need for agent having neuroprotective and neuropharmacological activity enhancing learning, memory and overall performance of the brain. [2] At the same time diabetes-induced learning and memory impairment is also at the rise.[3] *Moringa oleifera* (MO) plant parts are rich source of vitamins and antioxidants. They contain quantifiable amount of proteins, minerals, vitamin A, vitamin B complex, essential amino acids and a high content of vitamin E.[4] Traditionally this plant have been used for its memory facilitating effect.[5] Hence the aim of our research work was to evaluate the neurobeneficialeffect of the ethanolic extract of*Moringa oleifera*Lam podson diabetic stressed mice.

Materials and methods

Materials

The pods of *M.oleifera* were purchased from the local traders. Sample was authenticated by Head of the Botanical Department of Dr. BAMU University. An authenticated voucher specimen (No.0773) of the product has been preserved in same department for future reference. Metformin was obtained as a gift sample fromWockhardt , Aurangabad. Different chemicals like Petroleum ether (60- 80^0C), Ethanol 95% and STZ were purchased from Qualigens, Pune. All other chemicals used were of analytical grade.

Methodology

Preparation of ethanolic extract of *M. Oleifera* pods

The pods were dried under shade and powdered by using grinder mixer. The powdered material (150 g) was socked in Petroleum ether (60 – 80^0C) to remove lipids. It was further filtered and subjected for ethanolic extraction using soxhlet apparatus for 72hr. After extraction the solvent was evaporated using vacuum to obtain solid brown coloured dry mass.

Preparation of drug solution

Accurately weighed quantity of STZ (75 mg/kg) was dissolved in sufficient quantity of sterile saline water (0.9%).[6]Similarly, accurately weighed quantity Metformin (120mg/kg) was dispersed in distilled water.[7]Accurately

weighed quantity of ethanolic extract (100 and 200 mg/kg) was dissolved in the distilled water to prepare the appropriate stock solution of the extract. The doses were administered orally by selecting the appropriate concentration of the stock solutionas shown in table 1.

Table 1

Sr.no	Chemical	Dose	Route
1.	STZ	75 mg/kg	Intra-venous
2.	Glucose	5%	Oral
3.	Metformin	120 mg/kg	Oral
4.	Piracetam	100 mg/kg	Intra-peritoneal
5.	Ethanolic extract	100 mg/kg	Oral
6.	Ethanolic extract	200 mg/kg	Oral
7.	Diazepam	1 mg/kg	Intra-peritoneal

Animals

Swiss albino mice of either sex weighing between (25-40 g) were used. They were maintained at temperature of 25 ± 2˚C and relative humidity of 45 to 55% and under standard environmental conditions (12 hrs light /12 hrs dark cycles). The animals had free access to food and water. All the experiments were carried out between 9 to 18 hrs. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) of Y.B. Chavan College of Pharmacy Aurangabad(Approval number- CPCSEA/IAEC/P'col-20/2011-12/44).

Acute toxicity studies

Healthy adult albino mice (18- 22g) were subjected to acute toxicity studies as per guidelines (AOT 425) suggested by the organization for economic co-operation and development (OECD-2001). The mice were observed continuously for 2 h for behavioral and autonomic profiles and for any sign of toxicity or mortality.

Passive avoidance test

The equipment consist of electric grid (20 by 30 cm) with a shock free zone (SFZ) in the center and the entire grid having the perplex enclosure. Mice were kept individually on the electric grid and allowed to explore for about 1 min. The stimulus of 20 volt was given and latency of the animal to reach SFZ was recorded for 3 consecutive trials. Animals that reach SFZ in 2 minutes in first trial are to be preselected for the experiment after 24 hours of the first training. Each

animal is again tested for latency to reach SFZ and number of mistakes was calculated manually. 3 training trials were given to each mouse with 10-30 seconds interval.[8]

Elevated plus maze (EPM)

Locally fabricated elevated plus maze consisting of two open arms (35 × 6 cm) and two enclosed arms (35 × 6 × 15 cm) was used. The maze was elevated to the height of 40 cm. Mice were placed individually in the center of the EPM facing an enclosed arm. The time spent by the mouse during the next 5 min on the open and enclosed arm was recorded. The animals received vehicle (10 ml/ kg) or EMO (100, 200 mg/kg) 60 min before and diazepam (1 mg /kg intraperitonially) 30 min before their placement on the maze. Increased exploratory activity in the open arm was taken as an indication of anxiolytic activity.[9,10]

Object recognition test

The apparatus fabricated locally consisted of white colored plywood (70 × 60 ×30 cm) with a grid floor. It was illuminated by a 40 W lamp suspended 50 cm above the apparatus. The object to be discriminated was also made of plywood in two different shapes of 10 cm height and colored black. One day before the test, mice were allowed to explore the box without any object for 02 min. On the day of test, in the first trial (T1) conducted 60 min after administration of vehicle (10 ml/kg) or EMO (100,200 mg/kg) or piracetam (100 mg/kg) two identical objects were presented in opposite corners of the box and the time taken by each mouse to complete 20 s of object exploration was recorded (Exploration was considered as directing the nose at a distance less than 2 cm to the object and/or touching with nose). Second trial (T2) was performed 90 min after first (T1) and a new object replaced one of the objects presented in T1 and mice were left in the box for next 5 min. The time spent for exploring the familiar (F) and the new object (N) was recorded separately and discrimination index (D) was calculated as (N-F)/ (N+F), where N is time spent for new object and F is time spent for familiar object.The object was changed randomly and apparatus was cleaned with hydrogen peroxide after each trial to avoid place preference and the influence of olfactory stimuli respectively.[4]

Locomotor Activity

The locomotor activity (horizontal activity) was measured using a digital actophotometer (Space-lab, India). Each mouse was placed individually in the actophotometer for 5 min and basal activity score was obtained. Subsequently animals were divided into five groups and treated with test drugs. 60 min after dosing, the mice were placed again in the

actophotometer for recording the activity score as described earlier. The results were reported as mean change in the locomotor activity. Diazepam (2 mg/ kg, i p)preparation was used as reference standard.[11]

Statistical analysis

The results are expressed as mean + SEM**.** Comparison between the groups was made by one way analysis of variance (ANOVA) followed by Dunnett's test.

Results

Acute oral toxicity test

No mortality and no signs of any toxicity were evidence after the administration of a limit dose of 2000 and 3000 mg/kg ethanolic extract of *Moringa oleifera* in acute oral toxicity test hence, for oral administration the doses were selected as 100 mg/kg and 200 mg/kg.

Passive avoidance test

After 3 weeks of diabetes induction, cognitive dysfunction was observed. In passive avoidance test transfer latency to SFZ and number of mistakes were calculated. In a control/vehicle treated group both the parameters showed significant ($p<0.01$) lower as compare to diabetic, and diabetic stress group. After 3 weeks of respective treatment of Metformin 120, EMO 100, EMO 200 ,and Piracetam 100 mg/kg animals showed significant ($p<0.01$) decrease in transfer latency as well as number of mistakes.(Table 2, Fig. 1).

Table-2: Effect of EMO treatment on transfer latency to SFZ in passive avoidance test.

Group	Treatment	Transfer latency	No. of mistakes
A	Control	26.40 ± 1.27	4.66 ± 0.88
B	Diabetic control	68.42 ± 1.88*	10.83 ± 1.49
C	Diabetic + Stress(D+S)	77.63 ± 3.15**	11.66± 0.66**
D	D+S + Metformin (120mg/kg)	27.49 ± 1.25**	42.83± 0.60**
E	D+S + EMO(100mg/kg)	28.07 ± 1.85**	5.00 ± 0.57**
F	D+S + EMO(200mg/kg)	26.93± 0.75**	4.00± 0.57**
G	D+S+Piracetam(100mg/kg)	20.74±2.46**	2.50±0.34**

Results are expressed as Mean ± SEM (n=6). The data was analysed using One-way Analysis of Variance (ANOVA) followed by Dunnett's- test. * P<0.05, ** P<0.01 vs Respective diabetic stress.

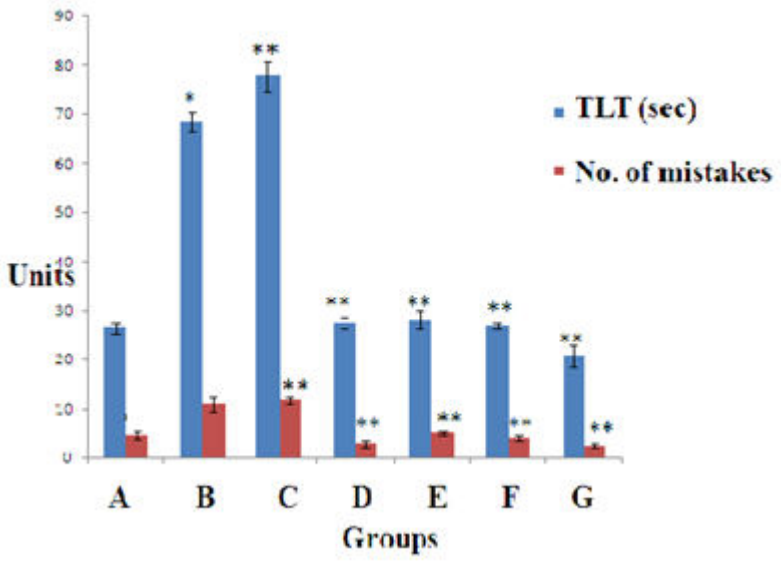

Fig. 1 Effect of EMO treatment on transfer latency to SFZ in passive avoidance test.

TLT: Transfer latency time.

Elevated plus maze

After 3 weeks of diabetes induction, cognitive dysfunction was observed. In EPM test transfer latency to closed arm were calculated on 1st, 2nd, and 7th day. In control group animal showed significant (p<0.01) decrease in the parameter such as Transfer Latency Time & number of mistakes when compared to STZ treated stress group.After 3 weeks of respective treatment of Metformin 120, EMO 100, EMO 200 and Piracetam 100 mg/kg animals showed significant (p<0.01) decrease in transfer latency as well as number of mistakes.(Table 3, Fig. 2) on 1st, 2nd ,and 7th day of treatment.

Table-3: Effect of EMO treatment on transfer latency time (TLT) to closed arm in EPM.

Group	Treatment	TLT DAY O	TLT DAY 1	TLT DAY 7
A	Control	59.85±0.97	24.62±0.44 **	23.98±2.35 **
B	Diabetic control	72.00±6.62	73.50±7.21	86.00±1.77
C	Diabetic + Stress (D+S)	71.83±6.67	78.55±2.93	85.50±1.91
D	D+S+ Metformin (120mg/kg)	58.73±2.60	31.66±6.67 **	21.00±3.50 **
E	D+S + EMO (100mg/kg)	53.01±5.75	33.00±4.27 **	27.83±4.66 **
F	D+S+ EMO (200mg/kg)	58.50±1.23	29.50±3.99 **	24.16±1.72 **
G	D+S+Piracetam (100mg/kg)	47.05±0.18	18.66±2.04 **	16.66±1.02 **

Results are expressed as Mean ± SEM (n=6). The data was analysed using One-way Analysis of Variance (ANOVA) followed by Dunnett's- test. * P<0.05, ** P<0.01 vs Respective diabetic stress.

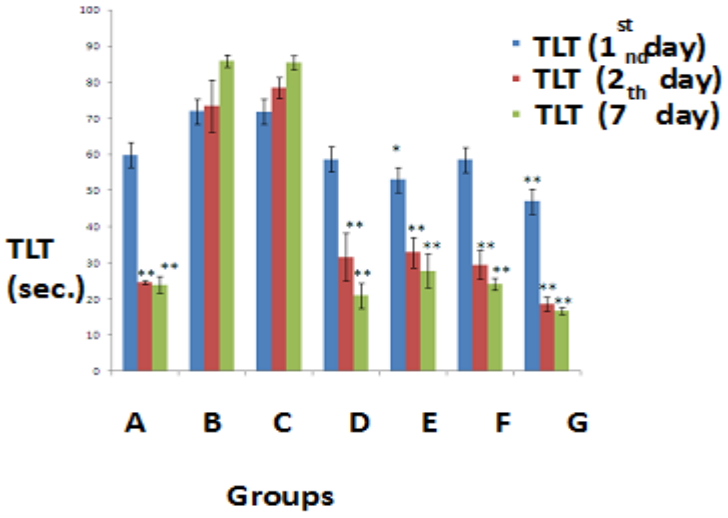

Fig. 2 Effect of EMO treatment on transfer latency to closed arm in EPM.

TLT: Transfer latency time.

Object Recognition

In object recognition test exploration time to familiar and novel object were calculated. In control group exploration to the novel object showed significant($p<0.05$) higher than familiar object where as in diabetic and diabetic stress group exploration to both object is equal. After 3 weeks of respective treatment of Metformin 120, EMO 100, EMO 200 and Piracetam 100 mg/kg animals showed significant ($p<0.01$) observation towards novel object compare to respective diabetic stress group. (Table 4, Fig. 3).

Table-4: Effect of EMO treatment on exploration to the novel object in object recognition test.

Group	Treatment	Exploration	
		Familiar object	Novel object
A	Control	9.38 ± 0.58	14.30 ± 1.51 *
B	Diabetic control	10.33±0.98	10.38 ± 1.00
C	Diabetic + Stress (D+S)	10.47±0.61	10.50 ± 0.61
D	D+S + Metformin (120mg/kg)	7.62 ± 0.58	14.08 ± 1.12*
E	D+S + EMO (100mg/kg)	9.40± 0.54	13.40 ± 0.56*
F	D+S+ EMO (200mg/kg)	9.75± 0.60	15.59 ± 0.92 **
G	D+S+ Piracetam (100mg/kg)	6.59±0.63	18.59±0.61 **

Results are expressed as Mean ± SEM (n=6). The data was analysed using One-way Analysis of Variance (ANOVA) followed by Dunnett's- test. * P<0.05 ,and **P < 0.01 vs respective diabetic stress group.

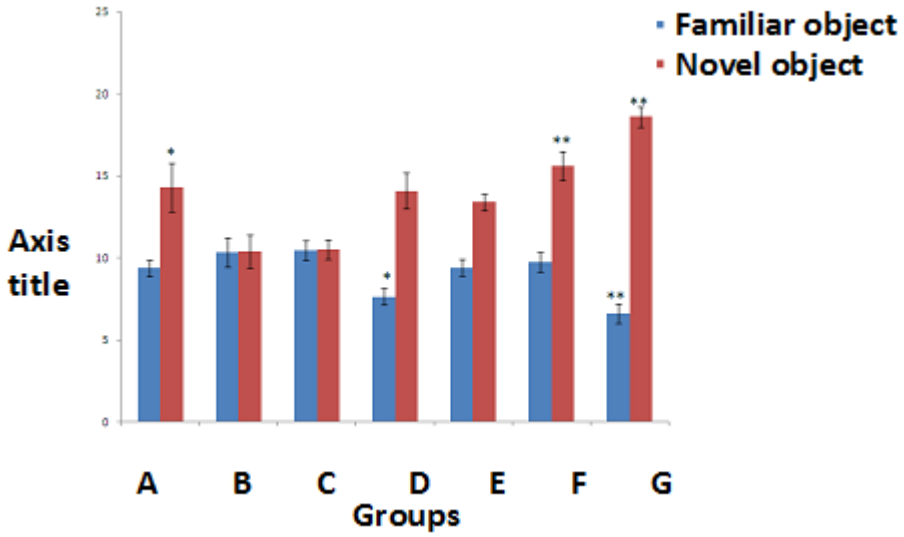

Fig. 3: Effect of EMO treatment on exploration to the novel object in object recognition test.

Effect of Locomotor activity

An EMO dose of 100 mg/kg did not produce any significant change in locomotor activity (16.66+2.88) as compared to control (10.16 + 1.70). However dose i.e. (200 mg/kg) produced significant increase (25.83+1.92) in locomotor activity.(Table 5)

Discussion

*Moringa oleifera*is traditionally claimed to be useful to improve learning and memory in Indian traditional system of medicine yet not documented scientifically in this regard .Despite extensive research, the neurological basis of learning and memory remains controversial. [12]

Hyperglycemia shows effect on locomotor activity in learning and memory.[13] It also shows reduced motor activity in diabetic animals.[14] Diabetes is related to a higher risk of amnestic mild cognitive impairment in a population with a high prevalence of this disorder like diabetic neuropathy, nephropathy and cognitive impairment. It may adversely affect the overall quality of life of the diabetic person both socially and economically and hence its management is very necessary.[15]

The conventional drugs used in the management of diabetic neuropathy and nephropathy are associated with a variety of side effects. When compared with well-defined synthetic drugs, herbal medicines exhibit some marked difference as are relatively safe and have lesser side effects in chronic diseases.

The Elevated Plus Maze test is suggested to be simple method for the evaluation of learning and memory processes. Since the animals are able to remember the configuration of the open and enclosed arms, they escape from the unsafe open arm more rapidly on the second trial. It is possible to evaluate the fear motivated learning, which underlies the transfer latency procedure in this test. Shortened transfer latency on second day's trial in mice is used as a parameter for retention or consolidation of memory, while treatment of drugs prior to first day may also be utilized for acquisition related action of drugs.

In object recognition test exploration of the animal to the novel object and familiar object is similar indicating memory impairment and it is observed in diabetic and diabetic stress mice. The improvement in discrimination index by the two doses (100 and 200 mg/kg) of extract proved the major criteria for nootropic activity.

The current study explored those findings demonstrating that diabetes reduced learning and memory performance. But Ethanolic extract of *M. oleifera*and Metformin treatment showed restoration of cognitive impairment.

Alzheimer's disease & Parkinson's disease are related to reduced learning and memory performance. The anticholinergic drugs useful to control Parkinson's disease may deteoriate cognitive behavior inco-existed Alzheimer's disease.[4] This complexity of various disorders of CNS may be due to widespread role of different central neurotransmitters and hence it is worthwhile to investigate the drug for detailed neuropharmacological actions.

Conclusion

It is thus apparent that ethanolic extract of *Moringa oleifera*pods exhibited decrease in transfer latency and number of mistakes in passive avoidance test and in EPM, improvement in the discrimination index, andincreased locomotor activity. These results suggest possiblerestoration of cognitive impairment by the extract.

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