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Research Article

Pharmacological Assessment Of Formononetin On Behavior, Cognitive Function And Oxidative Stress In Mice

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ABSTRACT

Introduction: This study was designed to investigate the chronic effects of Formononetin on behavior; cognitive function and oxidative stress in mice. **Materials and Methods:** Male albino mice of either sex (25 – 30 gm) were used in the study. The mice were divided into six different groups (n=6). Group 1: Control group received water. Group II: Mice received 5mg/kg/day, p.o. Formononetin for 21 days. Group III: Mice received 20mg/kg/day, p.o. Formononetin for 21 days. Group IV: Mice received 50mg/kg/day, p.o. Formononetin for 21 days. Group V: Mice received Diazepam 1mg/kg/day, I.P. Group VI: Mice received Imipramine 10mg/kg/day, p.o. Group VII: Mice received Piracetam 75mg/kg/day, I.P. **Result:** Chronic administration of FMNT at dose ranges between (5, 20,50mg/kg/day) revealed that significantly increased the time spent in open arm and decrease the no of entries in close arm in EPM showed Anxiolytic effect and also decreased the no of entries in dark compartment and increases the time spent in light compartment and also shows locomotor activity by increased no of square crossing in OF, whereas Antidepressant activity found by decreased immobility time in TST and FST and also find out the learning memory by NOR test which significantly express neuroprotective behaviour. The antioxidant parameters such as SOD, GSH and CAT level was found to be significantly increased in the FMNT as compared to the vehicle control groups. Level of SOD was found to be more in high dose of FMNT (50 mg/kg/ day). **Conclusion:** Our results suggest that chronic administration of FMNT can be observed and evaluated in laboratory mice. FMNT treated mice shown the anxiolytic, antidepressant and neuroprotective behavior such as increased time spent in open area, increased time spent in light area, decrease in immobility and improvement in memory. Improvement in memory is due to FMNT neuroprotective activity.

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INTRODUCTION

Formononetin is a naturally occurring molecule that is categorised as an isoflavone. It is widely distributed in many plant sources, especially legumes like soybeans (Glycine max), chickpeas (Cicer arietinum), and red clover (Trifolium pratense). Moreover, the anti-inflammatory and antioxidant activity of formononetin have been researched. Research has indicated that it can neutralise free radicals and lessen oxidative stress, shielding cells from harm. On the other hand, formononetin is the main bioactive component that carries out the neuroprotective effects [12].

The World Health Organisation estimates that 450 million individuals worldwide suffer from a mental or behavioural illness. Among mental illnesses, anxiety and depression disorders are the most common. By 2020, it is expected to rank as the second most common cause of disease worldwide. Anxiety is a mental, emotional, physical, and behavioural state that is both physiological and psychological. Anxiety can lead to feelings of tension, dread, fear, concern, and uneasiness as well as a lack of focus [2].

Depression is a multifaceted illness that frequently presents with behavioural, physiological, and psychological symptoms. It is a common mental illness that manifests as low energy, a gloomy mood, a loss of interest or pleasure, a feeling of guilt or low self-worth, trouble sleeping or eating, and difficulty concentrating [4 – 5].

Serotonin, Norepinephrine, Dopamine, GABA, Glutamate these neurotransmitters are targets for various treatments, including medications and therapies, to help manage anxiety and depression [11].

MATERIALS AND METHODS

Animals

Male albino mice of either sex (25 - 30 gm) were used in the study. The animals were procured from Lacsmi Biofarms, Pune, India. Mice were placed separately in polypropylene cages (n=6) per cage with paddy husk as bedding. The animals were maintained under standard laboratory conditions at temperature 25 ± 20 C, relative humidity 45 to 55 ± 10% and Light and dark 12 hrs cycles throughout the experiments. Animals had free access of water and standard laboratory feed. The animals were shifted in the laboratory one hour prior to the experiment .All the experiments were carried out between 10:00 am – 6:00 pm. The institutional animal ethical committee (IAEC) approved the protocol of this study (SSDJ/IAEC/22-23/03).

Experimental Design:

The mice were divided into six different groups (n=6).

Group 1: Control

Group II: Mice received 5mg/kg/day, p.o. Formononetin for 21 days.

Group III: Mice received 20mg/kg/day, p.o. Formononetin for 21 days.

Group IV: Mice received 50mg/kg/day, p.o. Formononetin for 21 days.

Group V: Mice received Diazepam 1mg/kg/day, I.P

Group VI: Mice received Imipramine 10mg/kg/day, p.o.

Group VII: Mice received Piracetam 75mg/kg/day, I.P.

Behavioural Parameters

At 21st day behavioural Parameters like Open Field Test [0FT], Dark and Light, Elevated plus Maze [EPM], Force Swim Test [FST], Tail Suspension Test [TST] were noted. Assessment of Cognitive function and Recognition memory in mice using Novel Object Recognition Test [NOR] and Memory Learning Test using EPM model was evaluated.

Tissue Homogenization

Mice were decapitated at the conclusion of the experiment, and the isolated brain was quickly washed with ice-cold Tris HCl buffered saline (pH 7.4). The brain was immediately blotted on filter paper after being cross-sliced into thin slices using

a surgical scalpel and suspended in a cold 0.25M sucrose solution. After that, the tissues were chopped and mixed thoroughly in cold Tris. Hydrochloric buffer (10 mM, pH 7.4) at a 10% w/v concentration. The goal of prolonged homogenization under hypnosis was to release soluble proteins by fracturing the cell's structure as much as possible. The homogenate was centrifuged using high speed cooling centrifugation for 15 minutes at 10,000 rpm and 0°C. The clear supernatant was used for the determination of superoxide dismutase (SOD), reduced glutathione (GSH), and catalase (CAT).

Open Field Test [OFT]

Animals were individually placed at one corner square of the open field. Animals were observed during 5 min. The parameters were noted- Ambulation (number of squares crossed), Rearing, Grooming.

Dark and Light

Place the animal gently in the centre of the dark chamber or at the doorway between the two chambers. Observe and record the animal's behavior for a set period, usually 5 minutes. Total time spent in the light and dark chambers and the number of times the animal crosses between the two chambers was recorded.

Elevated Plus Maze [EPM]

The animals were individually placed at the centre of the plus maze and observed for 5 min. The number of entries and time in seconds, spent by the animals in the open arm and closed arm were noted.

Force Swim Test [FST]

The animals are forced to swim in a cylinder (40cm x 18cm) with no escape filled with water temp. 25°C. Observe and record the animal's behavior for a set period, usually 6 minutes. First 2 Minutes: Often considered an acclimation period and not included in the analysis. Last 4 Minutes: Primary observation period where immobility and active behaviors are measured.

Tail Suspension Test [TST]

Each animal was suspended by the tail on the middle of a rod 50 cm above the floor using adhesive tape placed approximately 1cm from the tip of the tail. Immobility was recorded during a 6 min reading from first 2 min was discarded. Animals were considered to be immobile when it did not show any movement of body and hanged passively.

Novel Object Recognition Test

The experiments were carried out in an open field chamber made of non- transparent plexiglass. The NOR procedure consisted of habituation, acquisition and test phases. In the habituation phase, the mice were placed in an open field for 10 minutes without any object and subjected to an acquisition phase following an interval of 24 hrs. The acquisition phase consists of placing the mice in open-field arena with two identical sample objects (A+A) for 5 min. The test phase was performed after 90 minutes, where animal returned to open-field arena with two objects: the previously experienced sample object and a novel object (A+B), which the mice explored again for 5 min, as previously described (Espinosa et al., 2013). Exploration was defined as directing the nose to object at a distance equal to or less than 2 cm and/or touching it with the nose. Animals that did not investigate one or both objects during the sample phase were excluded. Results were analyzed as a discrimination index (DI) defined by the difference of time spent exploring the novel and the familiar objects in the test phase, over the total time spent exploring both objects, DI (BA)/(A + B). DI can vary between +1 and-1, where a positive score indicates more time spent investigating the novel object (preserved object recognition ability), whereas a negative score indicates more time spent investigating the familiar object, and a zero score indicates a null preference.

Assessment of Superoxide dismutase (SOD).

After diluting 0.5 millilitre of tissue homogenate with 0.5 millilitre of distilled water, 0.15 millilitre of ice-cold chloroform and 0.25 millilitre of ice-cold ethanol were added. After thoroughly mixing the mixture, centrifuge it for 15 minutes at 2500 rpm. Then 0.5ml of supernatant was mixed with 1.5ml of carbonate buffer and 0.5ml of EDTA solution. 0.4 millilitres of epinephrine was added to start the reaction, and the optical density change per minute at 480 nm was recorded in comparison to a blank. SOD concentration was expressed as units/ml. tissue. Change in optical density per minute at 50% inhibition of Epinephrine to adrenochrome transition the enzyme is taken units. Prepared the calibration curve by 10-125 units of SOD.

Assessment of Glutathione (GSH)

Equal amounts of tissue homogenate (supernatant) and 20% TCA were mixed. After centrifuging the precipitated fraction, mix 0.25 ml of supernatant with 2 ml of DTNB reagent. The phosphate buffer was added to get the final volume up to 3 millilitres. At 412 nm, the colour developed was measured against a reagent blank. Standard glutathione from Sigma Chemicals, St. Louis, MO, USA, was used to generate a graph. Lowry C and Folin's phenol reagent were used to measure the amount of protein in the spleen. The micrograms of GSH per milligram of protein was the unit of measurement.

Assessment of catalase (CAT)

Mixed 2ml diluted sample with 1ml of Hydrogen peroxide to initiate the reaction. Prepared the blank by adding 2ml of diluted sample in 1ml of phosphate buffer (50 mM. pH 7.0). The dilution should be such that the initial absorbance should be approximately 0.50. The decrease in absorbance was measured at 240nm.Catalase concentration was expressed as µmoles of H_2O, consumed/min/mg/protein.

RESULTS

Assessment of Anxiolytic activity

Effect of Chronic administration of Formononetin on EPM

Chronic administration of FMNT (5, 20 and 50mg/kg/day) showed significantly (75.83±2.89*, 95.7±7.51**) increased in open arm time in EPM as compared to vehicle treated group. Mice treated with standard (Diazepam) showed significantly (120.5±9.16***) increased in open arm time in EPM as compared to vehicle treated group.

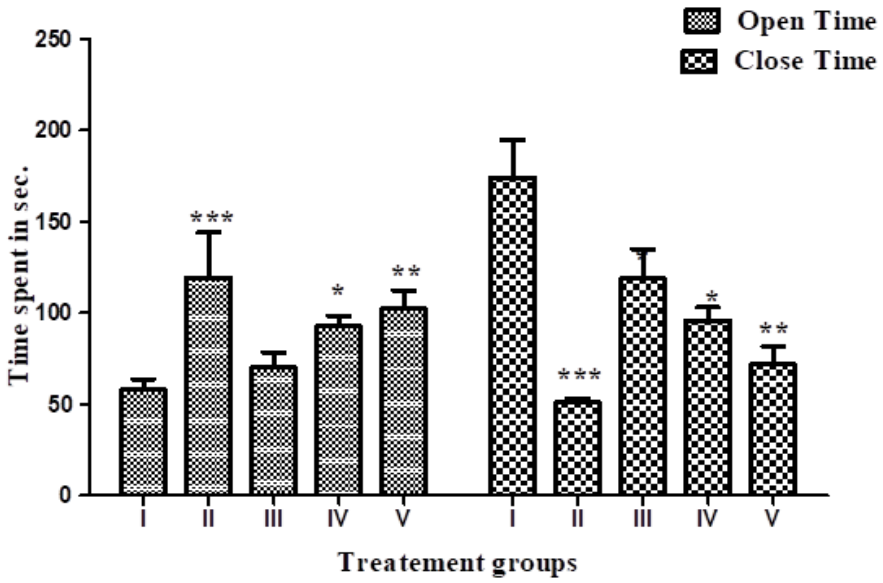

Figure 1: Effect of chronic administration of Formononetin on time spent in EPM

Effect of chronic administration of Formononetin in LDT

Chronic administration of FMNT (5, 20 and 50mg/kg/day) showed significantly (97.17±3.13, 97.67±6.24 and 105.7±13.92) increased the time spent in light compartment as compare to vehicle treated group. Mice treated with standard (Diazepam) resulted in (98.67±8.36) decreased in time spent in dark compartment as compared to vehicle control group.

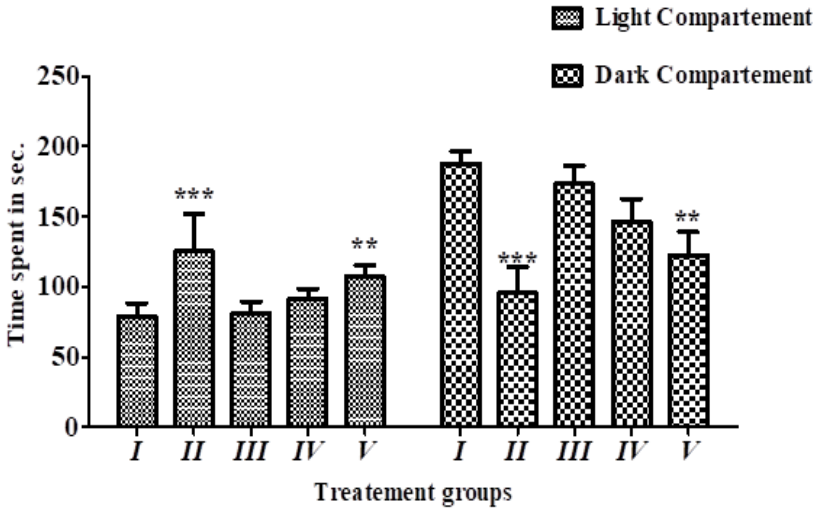

Figure 2: Effect of chronic administration of Formononetin in LDT

Assessment of locomotor activity by OFT

Chronic administration of FMNT showed significant (P<0.05) increase in no of square crossing as compared to vehicle treated group. It was noted that more pronounced effect at dose 50mg/kg of FMNT (85.7+13.92**) increases no of square crossing in OFT. Another group treated with standard drug (Diazepam) 1mg/kg IP it was significantly increase no of square crossing (101.33+61.41***) as compared to the vehicle control group.

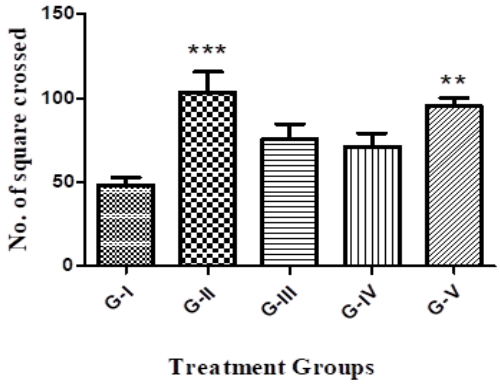

Figure 3: Effect of chronic administration of Formononetin on locomotor activity in mice by using OFT

Assessment of Antidepressant activity in mice

Effect of chronic administration of FMNT in TST

Chronic administration of FMNT resulted in more significantly increased duration of immobility at dosed 50mg/kg (105.7±13.92**) in TST using mice in comparison to the vehicle treated group. And standard group treated with (Imipramine) showed significantly (105.33±6.41**) decreased duration of immobility as compared to the vehicle treated group.

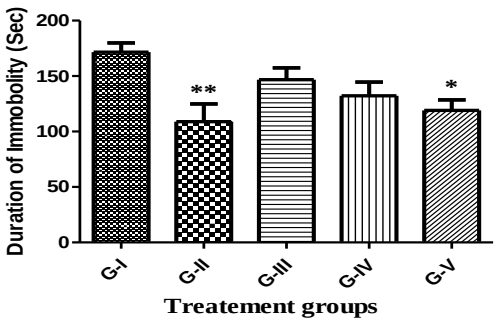

Figure 4: Effect of chronic administration of FMNT in TST

Effect of chronic administration of FMNT in FST

Chronic administration of FMNT at dosed (5, 20 and 50mg/kg) showed significant effect at 50mg /kg dose (114.27±4.92*) decreased in duration of immobility as compared to the vehicle treated group. One group treated with standard (Imipramine) it gives more significant (103.33±11.30**) decreased duration of immobility it was compared with vehicle treated group.

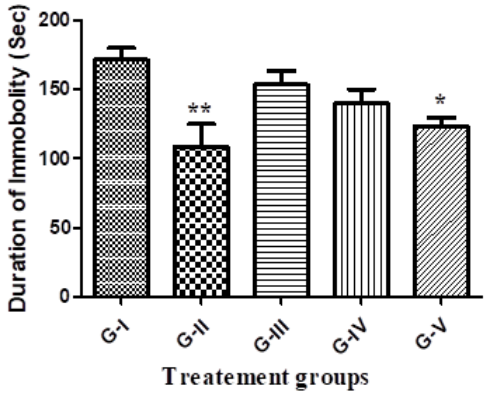

Figure 5: Effect of chronic administration of FMNT in FST

Assessment of Cognitive function and Recognition memory in mice

Effect of chronic administration of FMNT assessed the Recognition memory by using NOR test

Chronic administration of FMNT at dosed (5, 20 and 50mg/kg/day) showed significantly decreased in time taken to explore and recognize the similar object. The mice at dosed 20 and 50mg/kg FMNT showed more significant (75.35±56*, 70.45±2.21**) to explore the novel object as compared to vehicle treated group. Mice treated with standard (Piracetam) decreased the discrimination index (67.45±5.12***) as compared to vehicle treated group.

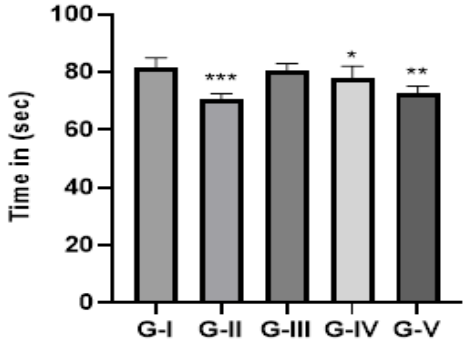

Figure 6: Effect of chronic administration of FMNT on Recognition memory activity by using NOR test.

Assessment of learning memory by using EPM Model

Chronic administration of Formononetin at different doses (5, 20,50mg/kg/day). Our research revealed that 20 and 50mg group significantly reduced (63.15 ± 5.34*, 60.67 ± 5.23**) latency time in the Plus maze apparatus as compared to the vehicle control group. Another group treated with standard drug Piracetam which gives better improvement (55.66 ± 4.37***) in learning memory as compared to normal group.

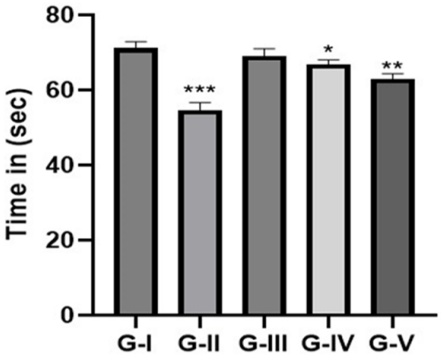

Figure 7: Effect of chronic administration of FMNT on learning memory by using EPM model

Effect of chronic administration of FMNT on Antioxidant properties parameter.

Effect of chronic administration of FMNT on Reduced Glutathione (GSH) Level

Chronic administration of FMNT showed significant ($p<0.001$) increased level of GSH in Diazepam, Piracetam and Imipramine as compared to control group. Whereas in groups

treated with FMNT 20 and 50mg/kg/day showed significant (p<0.001) increase in GSH level as compared to control groups.

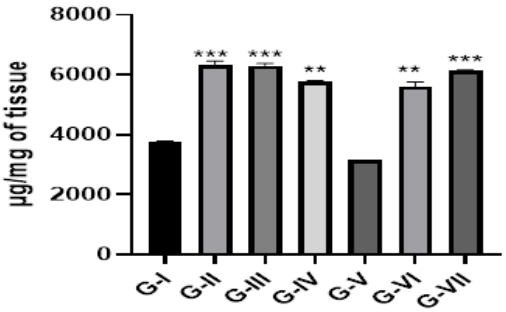

Figure 8: Effect of chronic administration of FMNT on GSH Level

Effect of chronic administration of FMNT on Superoxide Dismutase (SOD) level

The level of SOD was also monitored in all groups. Chronic administration of FMNT showed significant (p<0.001) increased level of SOD in Diazepam, Piracetam and Imipramine as compared to control group. Treatment with FMNT 20mg/kg/day showed significant (P<0.01) increased in SOD level and treatment with FMNT 50mg/kg/day showed better improvement in SOD level as compared to control group.

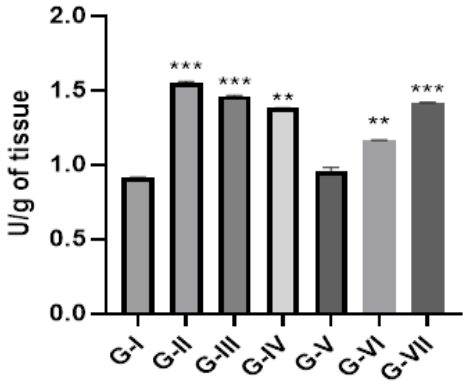

Figure 9: Effect of chronic administration of FMNT on SOD Level

Effect of chronic administration of FMNT on Catalase (CAT) Level

Chronic administration of FMNT showed significant (p<0.001) increased level of CAT in Diazepam, Piracetam and Imipramine treated group as compared to control group. Treatment with FMNT 20mg/kg/day showed significant (P<0.01) increased in CAT level and treatment with FMNT 50mg/kg/day showed better improvement in CAT level as compared to control group.

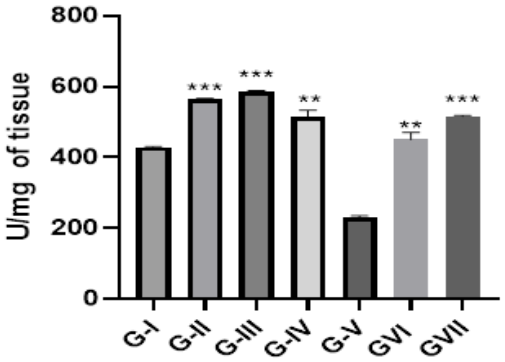

Figure 10: Effect of chronic administration of FMNT on CAT level

DISCUSSION

The entries into and the time spent in the safe environment relative to the risky environment are used as an index of animals stress level (an increase in exploratory behaviours toward and into risky environment indicate a relatively low level of stress). The number of paradigms including the elevated plus maze (composed of safer closed, dark arms verses riskier open bright arms), open field (consisting of darker wall- bordered peripheral portion versus a brighter open centre section), and light-dark transition box. This tendency is suppressed by anxiolytic and potentiated by anxiogenic agents.

In present study Chronic administration of FMNT (5, 20 and 50mg/kg/day) showed significantly (75.83±2.89*, 95.7±7.51**) increase in open arm time in EPM as compared to vehicle treated group. Mice treated with standard (Diazepam) showed significantly (120.5±9.16***) increase in open arm time in EPM as compared to vehicle treated group. In the EPM typical anxiolytic drugs show increased no. of entries and time spent in the open arms.

LDT is useful to study the anxiolytic activity. The animals latency to the dark chamber was decreased total transitions between compartments and the amount of time spent (sec.) in the light chamber significantly increased in the current investigation. In the present study the Chronic administration of FMNT (5, 20 and 50mg/kg/day) showed significantly (97.17±3.13, 97.67±6.24 and 105.7±13.92) increased the time spent in light compartment as compare to vehicle treated group. Mice treated with standard (Diazepam) resulted in (98.67±8.36) decreased in time spent in dark compartment as compared to vehicle control group. The tail suspension test a behavioral tests in rodent that predicted the clinical efficacy of many types of antidepressant treatments. In these tests, animals are under stress from which they cannot escape, after an initial period of struggling, they would become immobile, resembling a state of mental depression. By analogy with the behavior observed during the behavioral despair test rodent suspended by the tail rapidly become immobile after unsuccessful attempts to escape. Tail suspension test originally developed in mice.

In the present study Chronic administration of FMNT resulted in more significantly increased duration of immobility at dosed 50mg/kg (105.7±13.92**) in TST using mice in comparison to the vehicle treated group. And standard group treated with (Imipramine) showed significantly (105.33±6.41**) decreased duration of immobility as compared to the vehicle treated group.

The novel object recognition (NOR) test is another commonly used behavioral assay in pharmacology and neuroscience research, particularly for studying memory and cognitive function in rodents like mice. This test assesses the innate preference of rodents for novelty and their ability to recognize previously encountered objects.

Chronic administration of FMNT at dosed (5, 20 and 50mg/kg/day) showed significantly decreased in time taken to explore and recognize the similar object. The mice at dosed 20 and 50mg/kg FMNT showed more significant (75.35±56*, 70.45±2.21**) to explore the novel object as compared to vehicle treated group. Mice treated with standard (Piracetam) decreased the discrimination index (67.45±5.12***) as compared to vehicle treated group.

CONCLUSION

Our results suggest that chronic administration of FMNT shows anxiolytic activity in LDT and EPM by decreased no of entries in dark compartment of LDT and significantly increases time spent in open arm of EPM. FMNT shows antidepressant effect in TST and FST the immobility time was significantly shortened. In the OFT mice shows significantly locomotor activity by increased no of square. The recognition memory assessed by using NOR test in which mice significantly decreased in time taken to explore novel object, second test for learning memory it was performed in EPM by significantly shortened transfer latency as compared to the vehicle control group which revealed neuroprotective behaviour.

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