WORLD JOURNAL OF CURRENT MEDICAL AND PHARMACEUTICAL RESEARCH

www.wjcmpr.com ISSN: 2582-0222

In Vivo Assessment of Antidepressant upshot of *L - Fenchone* in Chronic Unpredictable Mild Stress (CUMS) induced depression like behaviour in Rodents

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ABSTRACT

A comprehensive approach like combining the natural medicine and CUS model for evaluating it as anti-depressant would be productive. Fenchone screened for anti-depressant activity in CUMS model using Fluoxetine as standard. Wistar albino rats were selected to CUMS procedure for 28 days and all the period test substance was administered at doses of 400 mg/kg and 800 mg/kg and at the end of the treatment behavioural and biochemical parameters were analyzed and histopathology findings were observed. CUMS exposure caused a depression like behavior corroborated by the increased immobility time in Despair swim test. In Actophotometer decreased locomotor activity and in the hole board test a decrease in the number of head dips and line crossings. Biochemical findings revealed that decreased serum oxide dismutase and catalase. Fenchone at the doses tested produced significant effects on behavioral and biochemical tests when compare to CUS group. These results manifested that Fenchone had specifically anti-depressant like effect in vivo. In conclusion, the present study advocate that the repeated administration of Fenchone notably reversed CUMS induced depression and oxidative damage and possessed antidepressant like effects, which would be of therapeutic interest for using Fenchone in the treatment of depressive disorders.

Key words:
Chronic Unpredictable Mild Stress, Despair Swim Test, Catalase, Super Oxide Dismutase, Fluoxetine.

Article History:
Received On: 29.06.2019,
Revised On: 31.10.2019,
Accepted On: 02.11.2019

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1. INTRODUCTION:

Depression is a long-standing malady that influences all age groups. Despite several efficacious antidepressants available today, the ongoing armamentarium of therapy is somewhat sparse, with undesirable results over one in three of all subjects treated[1].

Depression is indicated by disturbances in appetite and sleep, shortfall in cognition and energy. Thoughts of guilt, worthlessness, and suicide are common. Stroke and Coronary artery diseases are more customary in patients having depression, and depression may deliberately aggravate the prediction for patients with a diversity of concomitant aesculapain health[2].

Depression is distinguished by fierce dejection and desolation, dropping of attentiveness, melancholic worry, deficit pleasure and self-deprecation. Physical changes may occur, significantly in awful depression, including: insomnia, irregular devour patterns, with anorexia and slim down or sporadically overindulging; reduced energy and sex drive; and interference of the normal circadian and ultradian rhythms , body transposition, and multitudinous endocrine functions[3].

Even though a maximum number of antidepressants available, 30% to 40% of patients having depression disappoint to acknowledge the first-line antidepressant treatment[4].

Present day, natural medicines, with their high safety margins, had demonstrated to be productive pharmacotherapy in treating the depression. The model necessitates exposing the rat at unpredictable chronic stress for 28 days to a line of minor-intensity stressors. This outcome in the improvement of an integer of psychological alterations in majority of animals (some animals can be more stress-resistant), including anhedonia and apathy. This model is considered as good model for antidepressant action[5].

1.1HYPOTHESIS OF DEPRESSION[6]

- The receptor sensitivity theory/hypothesis
- Monoamine Theory
- The serotonin only hypothesis
- The latitudinarian hypothesis
- The Electrolyte membrane hypothesis

Stress is a physical rejoinder mechanism but the body has limited capacity to respond[7]

FENCHONE:

SYNONYMS:

1, 3, 3-Trimethyl-2-norbornanone. 1, 3, 3-Trimethyl-2-norcamphanone

SYSTEMATIC NAME:

1, 3, 3-Trimethylbicyclo [2.2.1] heptan-2-one

CHEMICAL CLASS:

Bicyclic monoterpenoid ketone

Note: many isomers of Fenchone available, and the remaining isomer-specific intimation has been specified: (1R, 4S)-(S)-Fenchone, CAS number: 7787-20-4

(1R, 4S)-(R)-Fenchone, CAS number: 4695-62-9

fig.1: (-)-fenchone

SOURCES: >1.0%

- Lavender (Spanish) 14.9–49.1%; Fennel (bitter) 4.0–24.0%; Thuja 12.2–12.8%; Fennel (sweet) 0.2–8.0%; Cedarwood (Port Orford) 4.7%; Labdanum 1.4–2.3%; Pine (Scots) 0–2.1%

ACUTE TOXICITY:

Oral LD50 in rats 6.16 g/kg and dermal LD50 in rabbits were tested for acute toxicity.

NEUROTOXICITY:

Fenchone was injected subcutaneously, developed clonic epilepsy in mice at 1,133 mg/kg, but not at 500 mg/kg. Given to dogs for 16 days orally, (þ)-Fenchone was convulsive and showed death at 1,400 mg/kg/day, produced mild jerking at 750 mg/kg/day, and had no significant effects at 210–420 mg/kg/day.

GENOTOXICITY AND MUTAGENICITY:

Fenchone was not genetically toxic and mutagenic. -Non mutagenic in Salmonella typhi strains, TA98, TA97, TA100 or TA1535, with or without S9 in rat bone marrow micronucleus test. While, Fenchone was not genetically toxic, though at a maximum dose (2,500 mg/kg ip for 3 days), the results were considered equivocal.

SUMMARY:

Fenchone seems to be non-allergenic, non-irritant and non-toxic[9].

2. AIM AND OBJECTIVE

AIM:

The present investigation is aimed to study the Antidepressant activity of Fenchone in chronic unpredictable stress induced depression like behaviour in rat model.

OBJECTIVE(S) OF THE STUDY:

To evaluate the Anti-depressant activity of Fenchone by estimating the following parameters:

- ✓ Behavioral parameters such as:
  - Spontaneous locomotor activity,
  - Sucrose preference test,
  - Despair swim test,
  - Hole board test
- ✓ Biochemical parameters like:
  - Superoxide dismutase (SOD)
  - Catalase (CAT)
- ✓ Histopathology of brain.

3. METHODOLOGY

3.1. ANIMALS:

Female Rats measure the weights of (200-220 g) were received from the veterinary college, Bangalore, acclimatized for two weeks before experimentation. Animals are placed 12 hour light/ dark cycle under constant humidity (50± 10%) and temperature (22 ±2^0 C) and animals were under normal pellet diet and water as much. Here all the experiments were performed between Mornings 10:00 to Evening 04:00. The experimental procedures on animals were in consent with the Committee for the Purpose of Control and Supervision of Experiments on Animals, CPCSEA (Approval Number 878/PO/Re/S/05/CPCSEA/003/2017).

3.2. DRUGS:

Fluoxetine was obtained from Glenmark Pharmaceuticals, India. (-)- Fenchone, SOD standard, Glutathione reduced, DTNB, Epinephrine bitartarate and Tris buffer were procured from Sigma-Aldrich, Bangalore, India. Disodium EDTA, Potassium Dihydrogen Phosphate, Sodium Chloride, sucrose, Sodium bicarbonate, Sodium carbonate, Hydrogen peroxide, Trichloroacetic acid, Sodium phosphate, formaldehyde, Normal saline and hydrochloric acid were obtained from different agencies.

3.3. DRUG TREATMENT:

Fluoxetine, a selective serotonin reuptake inhibitor, used as positive control for antidepressant action. Five groups of six animals in each were assigned randomly: Vehicle control, CUMS plus Vehicle, CUMS plus Fenchone (400 mg/kg), CUMS plus Fenchone (800 mg/kg) and CUMS plus Fluoxetine (20 mg/kg). Fenchone and Fluoxetine are administered orally between 9:30 Am and 10:30 Am once per day for four weeks.

3.4. CHRONIC UNPREDICTABLE MILD STRESS MODEL:

Rats were divided into five groups. (1) Non-Stressed, Control group (2) Stressed, CUS group (3) CUS with Low dose of Test (4) CUS with High dose of Test and (5) CUS with Standard treatment, positive control (Fluoxetine 10 mg/ kg). Each group consisting of six animals (n=6) and every group is placed in individual cages. Control group rats were kept undisturbed in their home cages, where as rats in the CUS group were subjected to various stressors for 28 days (four weeks). The stressors include food deprivation, Water deprivation, over night illumination, Slant cage, cold water immersion, foot shock and forced swimming. To avoid monotony, rats were exposed to these stressors at different times on each day. Behavioral tests were begin at day 29 after 24 hours of last foot shock. After behavioral tests, rat brains were isolated for biochemical analysis and for histopathology[15].

Table 1: Protocol for Chronic Unpredictable Stress (CUS).

DAY/ STRESS	Food deprivation	Slant cage	Over- night illumination	Forced swimming	Water deprivation	Cold water immersion	Foot shock
MON	10:00 a.m ↓						
TUE	10:00 a.m	10:00 a.m ↓					
WED		10:00 a.m	06:00 p.m ↓				
THU			06:00 a.m	10:00 a.m ↓ 11:00 a.m			
FRI					10:00 a.m		
SAT					↓ 10:00 a.m	10:00 a.m ↓ 11:00 a.m	
SUN							10:00 a.m ↓ 11.00 a.m

3.5 BEHAVIORAL PARAMETERS:

3.5.1 Spontaneous Loco motor Activity:
3.5.2Sucrose preference test:
3.5.3 Despair swim test: [28].
3.5.4 Hole Board Test (HBT)

3.6. OXIDATIVE PARAMETERS IN BRAIN TISSUE HOMOGENATE:

3.6.1. SUPEROXIDE DISMUTASE (SOD)

SOD was estimated by the method of Fridovich and Misra (1967).

3.6.2. CATALASE (CAT):

Catalase was estimated by Hugo E. Aebi method, 1974.

3.7. HISTOPATHOLOGY:

3.8. STATISTICAL ANALYSIS

The outcomes are expressed as the mean ± SEM. Statistical evaluation was carried out by using one way ANOVA followed by Dunnett's Multiple Comparison Test. P<0.05 was considered to be significant.

4. RESULTS AND DISCUSSION

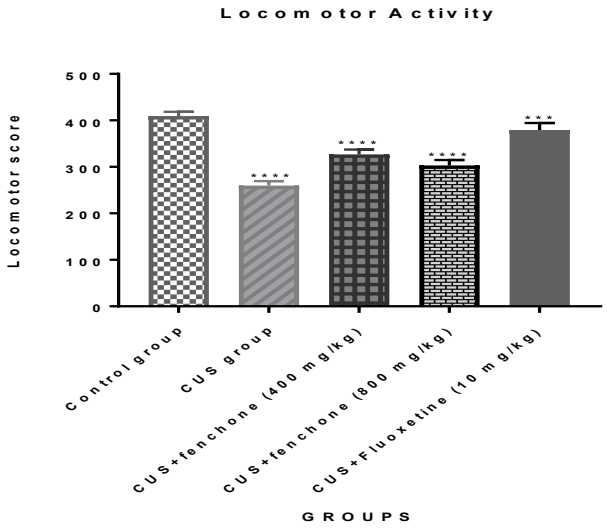
4.1. BEHAVIORAL PARAMETERS

4.1.1. SPONTANEOUS LOCOMOTOR ACTIVITY:

Table 2: Effect of fenchone on Locomotor Activity

S.NO	GROUPS	LOCOMOTOR SCORES
1	NORMAL CONTROL	409.5± 3.722
2	CUS GROUP	259.7±3.989****
3	CUS+ Fenchone (400 mg/kg)	303.3±4.745####
4	CUS+ Fenchone (800 mg/kg)	327± 40274####
5	CUS+ Fluoxetine (10 mg/kg)	379±6.129###

The values expressed are mean ± SEM, one way ANOVA followed by Dunnett's test. **** P < 0.0001 as compared with the control, #### P < 0.0001 as compared with the CUS group, and ### P < 0.05 as compared with the CUS group.



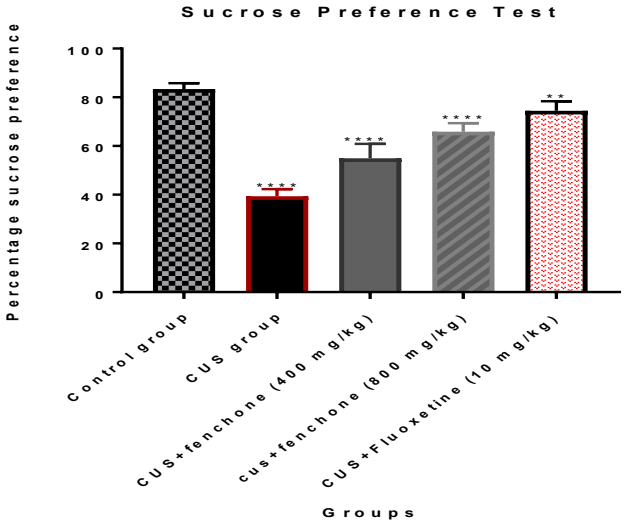
Graph 1: Effect of fenchone on locomotor activity in CUS induced rats was recorded. The values expressed are mean ± SEM, one way ANOVA followed by Dunnett's test. **** P < 0.0001 as compared with the control, , #### P < 0.0001 as compared with the CUS group and ### P < 0.05 as compared with the CUS group.

4.1.2. SUCROSE PREFERENCE TEST:

Table 3: Effect of fenchone on Sucrose Preference.

S.NO	GROUPS	%SUCROSE PREFERENCE
1	NORMAL CONTROL	83.85± 0.9839
2	CUS GROUP	39.38± 1.176****
3	CUS+ Fenchone (400 mg/kg)	54.93± 2.414####
4	CUS+ Fenchone (800 mg/kg)	65.86± 1.445####
5	CUS+ Fluoxetine (10 mg/kg)	74.42± 1.631##

The values expressed are mean ± SEM, one way ANOVA followed by Dunnett's test. ****P < 0.0001 as compared with the control, #### P < 0.0001 as compared with the CUS group and ## P < 0.05 as compared to CUS group.



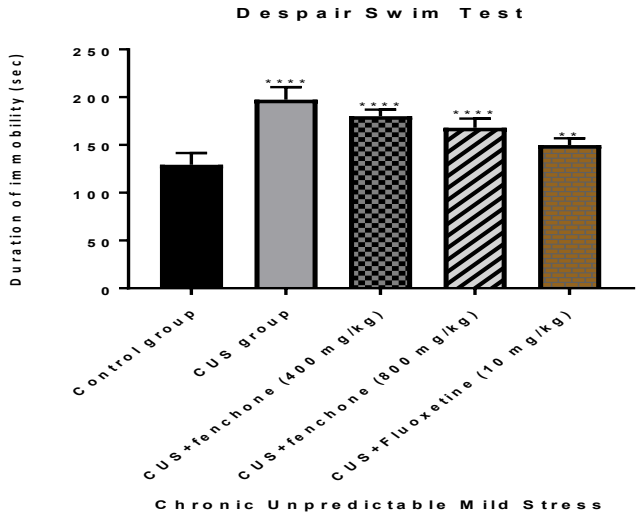
Graph 2: Effect of fenchone on sucrose preference in CUS induced rats was recorded. The values expressed are mean ± SEM, one way ANOVA followed by Dunnett's test. ****P < 0.0001 as compared with the control, #### P < 0.0001 as compared with the CUS group and ## P < 0.05 as compared to CUS group.

4.1.3. DESPAIR SWIM TEST:

Table 4: Effect of fenchone on Duration of Immobility.

S.NO	GROUPS	DURATION OF IMMOBILITY (Sec)
1	NORMAL CONTROL	129.3± 5.011
2	CUS GROUP	197.3± 5.408****
3	CUS+ Fenchone (400 mg/kg)	149.8± 2.892####
4	CUS+ Fenchone (800 mg/kg)	180± 2.852####
5	CUS+ Fluoxetine (10 mg/kg)	168± 3.933##

The values expressed are mean ± SEM, one way ANOVA followed by Dunnett's test. ****P < 0.0001 as compared with the control, #### P < 0.0001 as compared with the CUS group and ## P < 0.05 as compared with the CUS group.



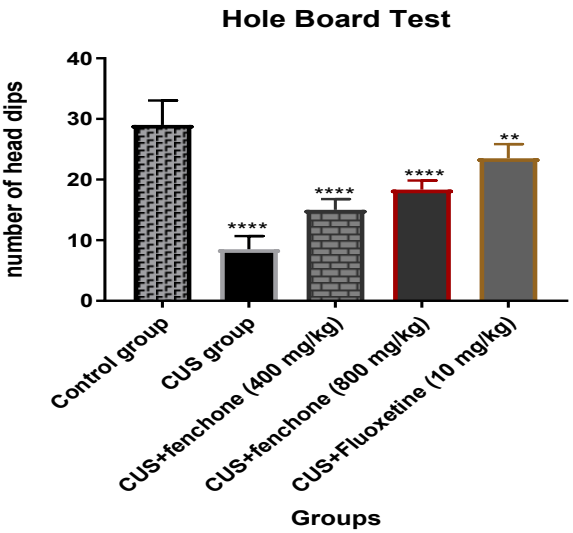
Graph 3: Effect of fenchone on duration of immobility in CUS induced rats was recorded. The values expressed are mean ± SEM, one way ANOVA followed by Dunnett's test. ****P < 0.0001 as compared with the control, #### P < 0.0001 as compared with the CUS group and ## P < 0.05 as compared with the CUS group.

4.1.4. HOLE BOARD TEST:

Table 5: Effect of fenchone on Hole board test.

S.NO	GROUPS	NUMBER OF HEAD DIPS (Sec)
1	NORMAL CONTROL	29 ± 1.653
2	CUS GROUP	8.5 ± 0.8851****
3	CUS+ Fenchone (400 mg/kg)	15 ± 0.7303####
4	CUS+ Fenchone (800 mg/kg)	18.33 ± 0.6146####
5	CUS+ Fluoxetine (10 mg/kg)	25.5 ± 0.9574##

The values expressed are mean ± SEM, one way ANOVA followed by Dunnett's test. ****P < 0.01 as compared with the control, #### P < 0.0001 as compared with the CUS group and ## P < 0.05 as compared to CUS group.



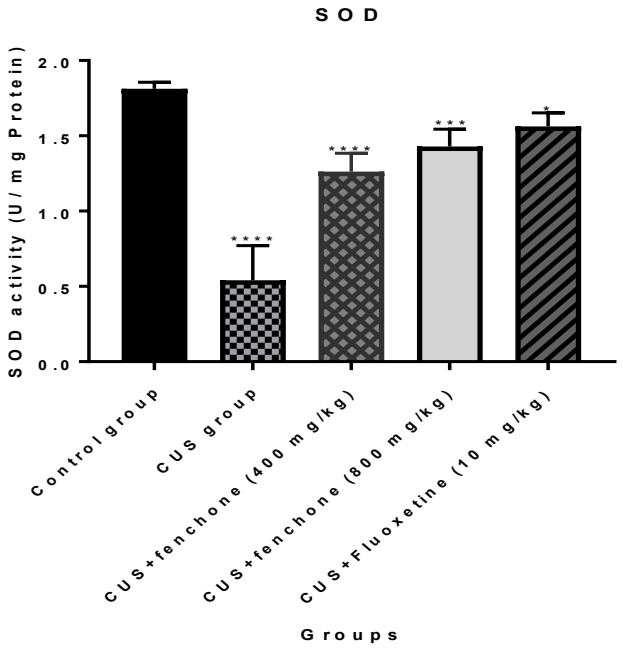
Graph 4: Effect of fenchone on number of head dips in CUS induced rats was recorded. The values expressed are mean ± SEM, one way ANOVA followed by Dunnett's test. ****P < 0.01 as compared with the control, #### P < 0.0001 as compared with the CUS group and ## P < 0.05 as compared to CUS group.

4.2.1. SUPEROXIDE DISMUTASE (SOD):

Table 6: Effect of fenchone on Superoxide Dismutase.

S.NO	GROUPS	SOD (U/mg Protein)	% of Control
1	NORMAL CONTROL	1.81 ± 0.017	100
2	CUS GROUP	0.54 ± 0.093****	29.38
3	CUS+ Fenchone (400 mg/kg)	1.26 ± 0.049####	69.61
4	CUS+ Fenchone (800 mg/kg)	1.43 ± 0.046###	79
5	CUS+ Fluoxetine (10 mg/kg)	1.56± 0.036#	86.18

The values expressed are mean ± SEM, one way ANOVA followed by Dunnett's test. ****P < 0.01as compared with the control, , #### P < 0.0001 as compared with the CUS group and # P < 0.05 as compared with the CUS group.



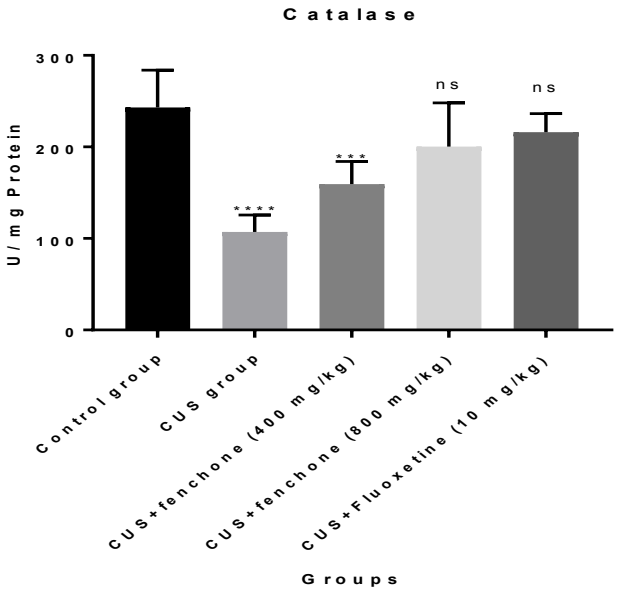
Graph 5: Effect of fenchone on SOD activity in CUS induced rats was recorded. The values expressed are mean ± SEM, one way ANOVA followed by Dunnett's test. ****P < 0.01as compared with the control,, #### P < 0.0001 as compared with the CUS group and # P < 0.05 as compared with the CUS group.

4.2.2. CATALASE (CAT):

Table 7: Effect of fenchone on catalase.

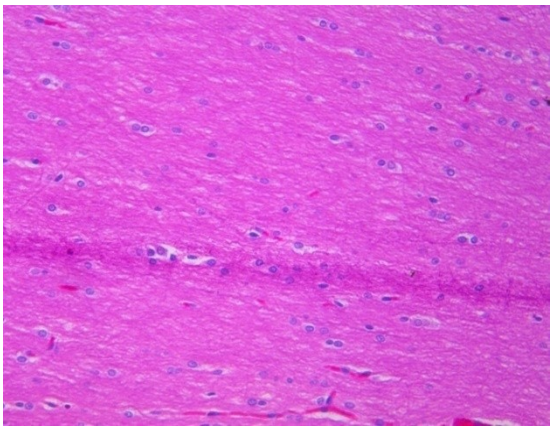
S.NO	GROUPS	Catalase (U/ mg Protein)	% of control
1	NORMAL CONTROL	243.1 ± 16.64	100
2	CUS GROUP	107.2 ± 7.546****	44.09
3	CUS+ Fenchone (400 mg/kg)	159.5 ± 10.07###	65.61
4	CUS+ Fenchone (800 mg/kg)	200.4 ± 19.48ns	82.43
5	CUS+ Fluoxetine (10 mg/kg)	216.1 ± 8.252ns	88.89

The values expressed are mean ± SEM, one way ANOVA followed by Dunnett's test.
****P < 0.0001 as compared to control, ### P < 0.001 as compared to CUS group and ns- non significant compared to CUS group.



Graph 6: Effect of fenchone on catalase activity in CUS induced rats was recorded. The values expressed are mean ± SEM, one way ANOVA followed by Dunnett's test.
****P < 0.0001 as compared to control, ### P < 0.0001 as compared with CUS group and ns- non- significant compared to CUS group.

4.3. HISTOPATHOLOGY



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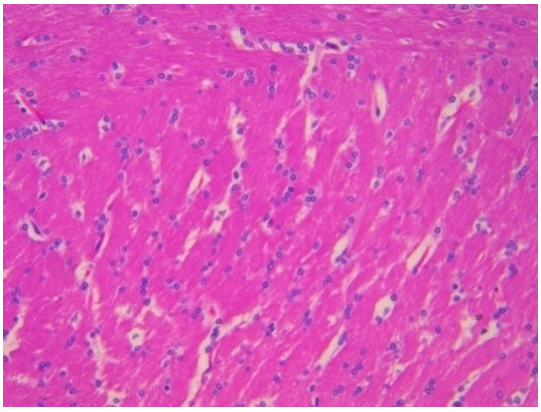

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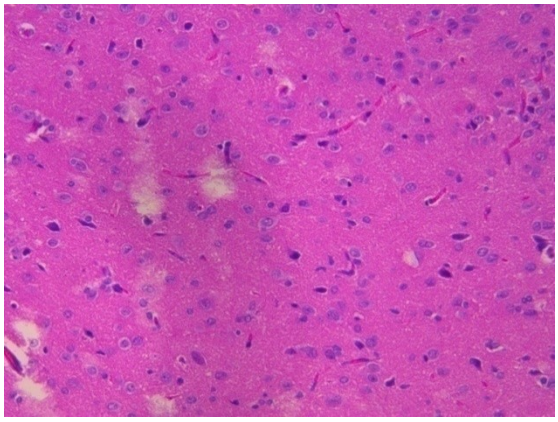

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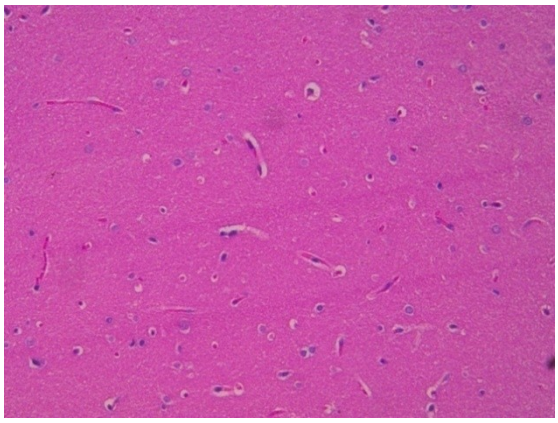

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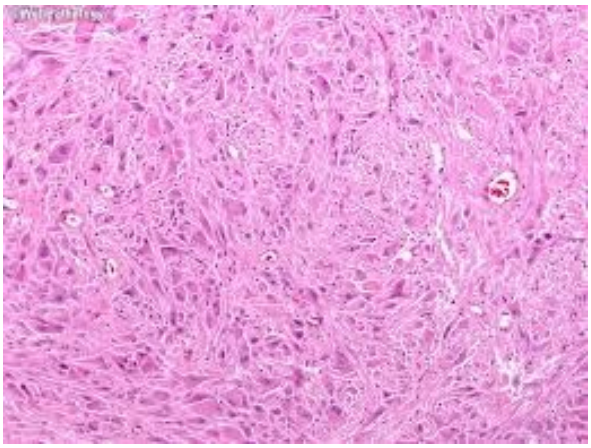

E

Fig.4.3: A. Control - Morphology of neurons in brain tissue was normal with no evidence of degenerative changes or inflammation.

Fig.4.3: B. Stress – Indicating several patchy neuronal degenerative changes with aggregation extensively dark pinocytic nuclei in neurons.

Fig.4.3: C. Fenchone 400 mg/ kg -Indicating patchy neuronal degenerative changes with dark pinocytic nuclei.

Fig.4.3: D. Fenchone 800 mg/ kg –Indicating mild degenerative changes and inflammation.

Fig.4.3: E. Fluoxetine 10 mg / kg – No significant degenerative changes or inflammation seen.

5. DISCUSSION:

It is normally trusted that Chronic Unpredictable Stress plays an important role in beginning and worsen of depression[29]. In this view, a model of CUMS induced depression has been initiated to stimulate the pathogenesis of depression in Homo sapiens. A number of investigations proposed that CUMS can result (in) long-term behavioral disturbances mimic manifestation of depression and that CUMS prompted depression model can be pre-owned for evaluating the effectiveness of antidepressants through behavioral tests like forced swim tests (FST) and sucrose preference Test (SPT). SPT is a measure of misery like behavioral changes, reduced consumption of sugary solutions. The investigations of current study manifest that rats exposed to CUMS procedure guzzle less sucrose solution when compared to normal rats. Chronic stress has been exhibited to dramatically increase the immobility time of rat in forced swim test, an exhibition of behavioral despair[30]. Unfailingly, CUMS, as observed in the current study, resulted in an increased immobility time in forced swim test in rats, decrease in number of head dips indicates Fenchone produced antidepressant like action in CUMS exposed rats.

6. CONCLUSION:

In conclusion, abiding fenchone treatment during the tack of CUMS was found to relive CUMS induced depression. Whether there is a central action of fenchone that it is critically salient for its anti-depressive effect will be further investigated.

7. LIST OF ABBREVIATIONS:

CAT: Catalase.
CUMS: Chronic Unpredictable Mild Stress.
DST: Despair Swim Test
HBT: Hole Board Test
SSRI: Selective Serotonin Reuptake Inhibitor.
SOD: Super Oxide Dismutase.

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