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Review Article

STUDY OF NEUROPROTECTIVE ACTIVITY OF SOME HERBAL DRUG EXTRACT

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As the brain ages, distinct alterations or events occur in neuron cell bodies which are associated with pathological lesions characteristics of neurodegenerative diseases. Various trace metals, copper, manganese, iron and other transition metals, excess glucocorticoid secretion may be involved in mediating these processes. To better understand the effects on the nerve cells specifically in hippocampus, a more experimental study on rodent mammalian model could be helpful. Taking this approach, a specific free swimming stress model to induce degenerative characteristics in hippocampal cells was used. A specific paradigm in male & female mice was used to investigate the impact of chronic stress exposure on the various characteristics such as number of nerve cell bodies in the hippocampal layers (CA1-CA4 & Dg) as well as nerve cell characteristics which include various degenerative changes before & after stress paradigm.

Key Words: Cinnamon oil, Hippocampal Cells, Nerve Cell.

INTRODUCTION:

"Behavior" is the response of an organism towards the environment or surroundings, governed by the dopaminergic transmission of the central nervous system. Disruption of this dopaminergic transmission will effect behavioral responses, which are related to the ability to "feel" the environment & to take decisions based upon those sensations, which will affect the emotional status of the individual, that is survival through the attribution of incentive salience to significant environmental stimuli & contextual reward/avoidance learning. [1,2] These behavioral responses are vital for the survival of the organism & are exhibited in varied forms by them, viz. exploration, fighting, aggression, feeding, sexual behavior etc. These traits are important to study as they form a base line of the survival of the organism including almost every activity & phase of its life.

In 1973, **Hans seyle** defined stress as "the non specific response of the body to any demands made on it". While more recent definitions have tended to see the stress response in terms of both a survival mechanism & an indicator of internal-external cues. [3] Stress is also manifested as a general reaction of the mammalian central nervous system which plays a vital role in the way an organism monitors internal conditions, as well as conditions in the world around it, in order to attempt to survive. [4] Thus the term stress is ambiguous & precludes accurate definition. Stress is a threat or challenge to the integrity & survival of the organism. [5] Stress is known to induce alteration in various physiological events leading to pathological state. Biological stress of varied types leads to general adaptation syndrome. Stress response is characterized by biochemical, physiological & behavioral changes in the body. Intense or chronic stress lead to the well known stress related diseases such as hypertension, diabetes, stroke, cancer, depression etc. as observed in human. [6]

Stress can have marked neutral & behavioral effects

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across mammalian species. Specifically, chronic exposure to stress can result in neural atrophy & associated memory deficits (e.g. learning & memory) in rodents [7,8,9,10]& humans. [11] Exposure to acute psychosocial stressors have been shown to suppress cell proliferation in the dentate gyrus of developing rats, [12], adult tree shrews [13]& adult marmoset monkeys [14]. A stressful stimulus can be considered as any event, which precipitates a significant activation of the sympathoadrenal medullary system & a measurable change in the behavior. [15] The different types of acute or chronic stressors in experimental animals include immobilization, tail shock, cold stress, forced swim stress etc. When animals were subjected to acute stress a wide range of physiological alterations take place. [16]

MATERIAL AND METHODS:

Animals :

Twenty four (24) Swiss albino mice of both sexes were used for the experiment (B.W.45±5 gm). Animals were purchased from Udaipur Veterinary College, Udaipur, Rajasthan. Mice were housed in polyutherine cages to acclimatize them to laboratory conditions. All the mice were given access to food & water, ab libitum & were maintained at 12:12 hr light & dark cycles at 27±2^{0}C. All animals were kept under these conditions for two weeks. After that mice were divided into control & experimental groups.

Treatment schedule

Control group :

Mice were (n=6) kept in pathogen free environment in isolated room. Rooms was locked for 24 hrs. This was necessary to avoid the stressful situation, which can be developed even in handling of animals or noise.

Experimental Groups :

In this group animals were divided into 2 experimental groups E1 & E2, each group having 6 animals.

1. E1 or STRESS GROUP

In this group mice (n=6) were given 3 hrs. swim stress daily for 30 days.

2. E2 or STRESS + DOSE

In this group mice (n=6) were given only Cinnamomum bark extract & floral extract of Clitoria sps. (Hydroalcoholic,70:30 ratio) for 30 days (40 mg/ kg/body wt.).

Stress Protocol:

All animals of E2 group were subjected to swim stress. For this animals were forced to swim for 3 hours specifically for 9:00 – 11:00 A.M. in luke water ranging from 25-27^{0}C for 30 days. Colonic /Rectal temperature was measured every day immediately before & after stress & other hyperactivities were also recorded for stress determination e.g. prostration, salivation, etc. Besides this daily record of food & water intake was also maintained for all the groups.

Drug Preparation:

The bark of Cinnamon were purchased from local supplier & flowers of Clitoria sps. were obtained from Rajasthan Agriculture College, Udaipur. Dried bark & flowers were purified using absorption method, by keeping them in contact with brick powder. After purification, bark & flowers were powdered finely, packed in high quality filter paper & extract was prepared by continuous extraction method with hydro-ethanol as solvent with the help of soxhlet extractor.

After vacuoevaporation crude extract was dissolved in aqueous medium (H_2O). **Diazepam** (3mg/kg i.p. for sedative effect) &**thiopental** ((60mg/kg i.p. for hypnotic effect) were used as positive control.

Dose Schedule:

The mice were given a daily drug extract dose 40 mg/kg of body weight after 11:00 A.M. i.e. after stress treatment. Dose was administered orally (using feeding tube). Oral administration of drug was done daily for one month period up to last day of stress treatment in E2 group.

Behavioral Analysis :

Three different types of behavioral analysis was performed which include :

(a) **Open field behavior test.**

(b) **Cateleptic behavior.**

(c) **Spatial task test.**

This study is conducted to analyze the reaction between time course of effect & the complementary effect of drug. So the observations were conducted at 1st, 7th, 14th, 21st& 28th day of the experiment. Each test was conducted after 11:00 AM, after stress treatment.

Hole Board Test (Open Field Behavior Test):

The hole board test **(HBT)** is an experimental method used in scientific research to measure anxiety, stress, neophilia & emotionality in animals. [35-37] Because of its ability to measure multiple behaviors, it is popular test in behavioral pharmacology but results are controversial. [38] The HBT was designed in the 1970's to mitigate the flaws of the open field test **(OFT).** Exploratory behavior requires a choice to be made to explore, but since the OFT is just simply a brightly lit area, the animal does not have a choice. The OFT does not differentiate between locomotion & exploration. [39]

APPARATUS : The hole board apparatus consisted of a wooden, grey box, measuring 68 X 68 cm. The walls were 40 cm high & the box was raised 28 cm above the ground on a metal stand. Sixteen (16) holes (4 cm in diameter) were cut into the floor of the apparatus, each hole was 28 cm from a corner of the box along the diagonal from the corners of the centre. The apparatus was located in a small testing room with dimmed white lighting. The stand of the apparatus was opened on all sides, allowing the floor or objects to be dimly lit.

METHOD : Mice were individually placed in the centre of a perforated board & number of head dips was registered during a time period of 5 minutes. The number of explored holes provides a measure of the number of head dips. [40]The result are given in the Table-1.

Fig.1: Hole Board Apparatus

ACTIVITY CAGE/ ACTOPHOTOMETER :

EQUIPMENT: It consist of six built in photosensor & four digital counter to indicate the locomotor activity. It measures then spontaneous & indicated activity with

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digital totalize. It also incorporates electric shock of up to 100 volts for activating rats. The stimulus is variable from 0-100 volts & indicating on meter.

PRINCIPLE :

Most of the CNS acting drugs influence the locomotor activities in man & animals. The CNS depressants drugs such as barbiturates & alcohol reduce the motor activity while the stimulants such as caffeine & amphitamines increase the activity. In other words, the locomotor activity can be index of wakefulness (alertness) of mental activity.

The locomotor activity (horizontal activity) can be easily measured using an actophotometer which operates on photoelectric cells which are connected in circuit with a counter. When the beam of light falling on the photo cell is cut off by the animal, a count is recorded. An actophotometer could have either circular or square arena in which the moves. Both rats or mice may be used for testing in this equipment.

PROCEDURE :

(1) Weigh the animals (20-25 gm) & number them.

(2) Turn on the equipment & placed individually each mouse in the activity cage for 10 minutes. Note the basal activity score of all the animals.

(3) Inject the drug thiopentyl (dose 3 mg/kg i.p.) & after 30 minutes re-test each mouse for activity score for 10 minutes. Note the difference in the activity before & after thiopentyl.

(4) Calculate percent decrease in motor activity.

METHOD:

The effect was recorded for disappearance (latency) & reappearance (duration) of the righting refex. Hypnotic sleeping time was considered to be the time interval between disappearance & reappearance of the righting reflex. [41] The result are given in the **Table-2**.

Fig. 2 : Actophotometer

TRACTION TEST (CATELEPTIC TEST) :

Mice were individually suspended by anterior limbs to a wire stretched horizontally. Abnormal mice that fails to make a re-establishment at least once of its posterior limbs to reach the wire are considered as subject under a sedative action. When the animals perform normal re-establishtment immediately, the reaction is known as positive; otherwise, the reaction is called negative; also the behavior of animals were recorded during the period of experiment.[42] The result are given in the **Table-1**.

SPATIAL TASK TEST :

For spatial task test a metallic rod of 1.25 m long & 1.25 cm diameter was kept at 1.25 ft. height. Rod was divided into 3 equal zones – 2 at periphery denoting fearful zone & no fear zone in middle region. Each animal was given 5 minutes time period for this test. Parameters studied during test were total time invested

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in moving between 2 ends, fall frequency, number of the motive to observe the change in balance behavior, its relation with fear as important by effect of the stress in animals & compared with those receiving drug as combating agent. Inferences drawn were correlated with histological hippocampus status observed in later experiment.

STATISTICAL ANALYSIS :

The statistical analysis was done using ANOVA. The results with P<0.05 were considered significant. The data are expressed as Mean ± S.D.

Group	Treatment	Dose (mg/kg)	Traction test (Re-establishment time in sec.s)	Hole-Board Test (Explore hole during 5 min.s)
III	HECZ-200	200	5.2±0.12	6±0.74
IV	HECZ-400	400	7.4±0.33	3.2±1.03

Table-1: Sedative action of test substance & standard drug in mice.

Group	Treatment	Dose (mg/kg)	Traction test (Re-establishment time in sec.s)	Hole-Board Test (Explore hole during 5 min.s)
I	Control	Vehicle	0.01±0.04	11±1
II	Only Diazepam	3	12±0.5	0.01±0.00
V	HECT-200	200	8.6±0.10	5.7±0.46
VI	HECT-400	400	9.8±0.21	2.8±0.12
VII	HECP-200	200	6.6±0.43	7.4±1.23
VIII	HECP-400	400	8.7±0.78	3.4±0.56

All values are represented as Mean±SD. (n=6)

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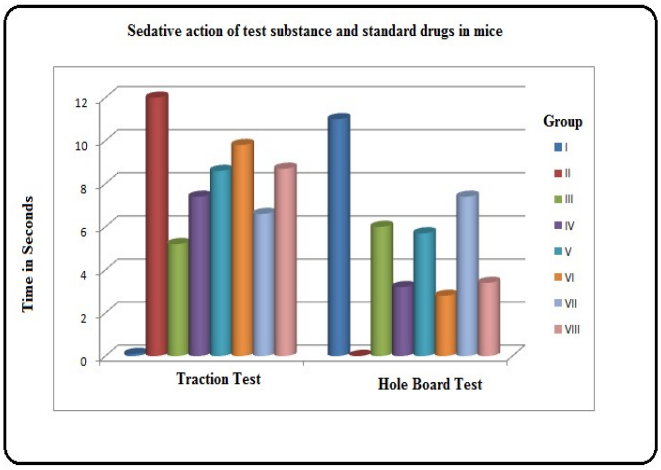

Diagram 1: Sedative action in Mice

Table-2: Hypnotic action of test substance & standard drug in mice (activity cage).

Group	Treatment	Dose (mg/kg)	Sleep Latency (Time in min.s)	Sleeping time (Time in min.s)
I	Control	Vehicle	5±0.51	32±2.01
II	Only Thiopentyl	60	13±0.31	81±1.03
III	HECZ-200	200	7±0.29	46±0.71
IV	HECZ-400	400	11±0.72	57±0.13
V	HECT-200	200	6.8±0.36	61±0.97
VI	HECT-400	400	9.7±0.19	48±2.09
VII	HECP-200	200	5.9±0.17	48±2.09
VIII	HECP-400	400	10.2±0.43	63±1.16

All values are represented as Mean±SD. (n=6)

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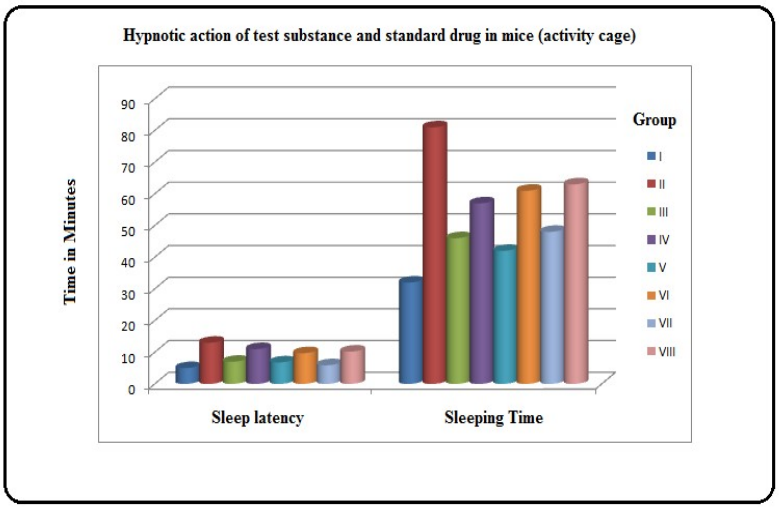

Diagram 2: Hypnotic action in Mice

Result :

Male swiss mice (20-25 gms) were used in pharmacological tests. The animals were feed ad libitum with standard food and water expect when fasting was required in the course of the study

Preparation of drug

All drugs and hydro alcoholic extracts of *Cinnamomum zeylanicum, Clitoria ternatea* and *Clitoria purpuria* were freshly prepared on the day of the experiments A control grops received distilled water (10 ml/kg, p.o.) as vehicle. Diazepam (3 mg/kg i.p. for sedative effect) and thiopental (60 mg/kg i.p. for hypnotic effect) were used as positive control

Material and Methods (Sedative and Hypnotic activity)

Traction Test

Mice were individually suspended by anterior limbs to a wire stretched horizontally. Abnormal mice that fails to make a reestablishment at least one of its posterior limbs to reach the wire are considered as subject under a sedative action. when the animals perform normal reestablishment immediately, the reaction is known as positive; otherwise, the reaction is called negative; also the behavior of animals were recorded during the period of the experiment[1]. The result are given in Table-1

Hole-Board Test

Mice were individually placed in the centre of a perforated board, and the number of head dips was registered during a 5 minutes. The perforated board test was made by using a wood floor board, 40 cm X 40 cm X 25 cm, in which evenly spaced holes were made. The number of explored holes provides a measure of the number of head dips[2]. The result are given in Table-1

Thiopental-Induced Sleep in Mice (activity cage)

The effect was recorded for disappearance (latency) and reappearance (duration) of the righting reflex. Hypnotic sleeping time was considered to be the time interval between disappearance and reappearance of the righting reflex[3]. The result are given in Table-2

Statistical Analysis The statistical analysis was done using ANOVA. The results with $P<0.05$ were considered significant. The data are expressed as

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Mean ± S.D.

The activity of hydro alcoholic (ratio of 70:30) extract of all three samples were studied

The group is as followed for the study

- ***Group I***- *Mice were given only vehicle.*
- ***Group II-*** *Mice were given Diazepam (3 mg/kg i.p.)*
- ***Group III-*** *Mice were given hydro alcoholic extract of Cinnamomum zeylanicum (200 mg/ kg/ bw, p.o.)*
- ***Group IV-*** *Mice were given hydro alcoholic extract of Cinnamomum zeylanicum (400 mg/ kg/ bw, p.o.)*
- ***Group V-*** *Mice were given hydro alcoholic extract of Clitoria ternatea(200 mg/ kg/ bw, p.o.)*
- ***Group VI-*** *Mice were given hydro alcoholic extract of Clitoria ternatea(400 mg/ kg/ bw, p.o.)*
- ***Group VII-*** *Mice were given hydro alcoholic extract of Clitoria purpuria(200 mg/ kg/ bw, p.o.)*
- ***Group VIII-*** *Mice were given hydro alcoholic extract of Clitoria purpuria (400 mg/ kg/ bw, p.o.)*

Table-1 Sedative action of test substance and standard drug in mice

Group	Treatment	Dose (mg/kg)	Traction Test (Re-establishment time in seconds)	Hole- Board Test (Explore hole during 5 minutes)
I	Control	Vehicle	0.10 ± 0.04	11 ± 1
II	Only Diazepam	3	12 ± 0.5	0.01 ± 0.00
III	HECZ- 200	200	5.2 ± 0.12	6 ± 0.74
IV	HECZ- 400	400	7.4 ± 0.33	3.2 ± 1.03
V	HECT-200	200	8.6 ± 0.10	5.7 ± 0.46
VI	HECT-400	400	9.8 ± 0.21	2.8 ± 0.12
VII	HECP-200	200	6.6 ± 0.43	7.4 ± 1.23
VIII	HECP-400	400	8,7 ± 0.78	3.4 ± 0.56

All values are represented as Mean ± SD (n=6)

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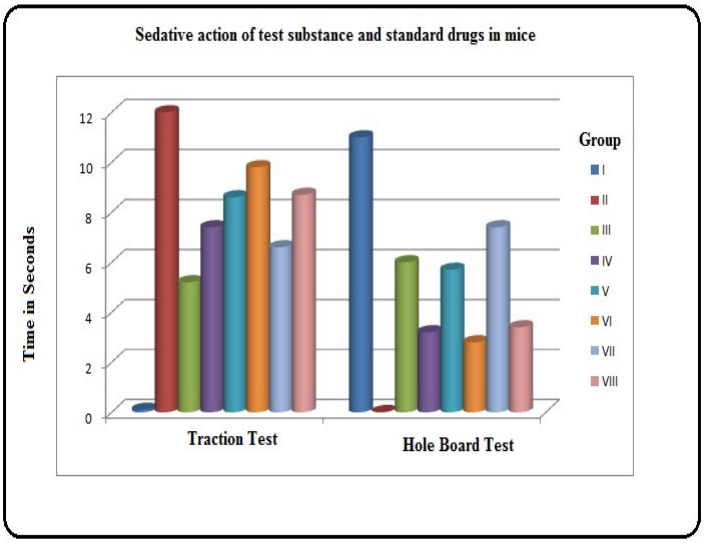

Diagram 3: Sedative action in Mice

Table-2 Hypnotic action of test substance and standard drug in mice (activity cage)

Group	Treatment	Dose (mg/kg)	Sleep latency (Time in minutes)	Sleeping time (Time in minutes)
I	Control	Vehicle	5 ± 0.51	32 ± 2.01
II	Only Diazepam	3	13 ± 0.31	81 ± 1.03
III	HECZ- 200	200	7 ± 0.29	46 ± 0.71
IV	HECZ- 400	400	11 ± 0.72	57 ± 0.13
V	HECT-200	200	6.8 ± 0.36	42 ± 0.89
VI	HECT-400	400	9.7 ± 0.19	61 ± 0.97
VII	HECP-200	200	5.9 ± 0.17	48 ± 2.09
VIII	HECP-400	400	10.2 ± 0.43	63 ± 1.16

All values are represented as Mean ± SD (n=6)

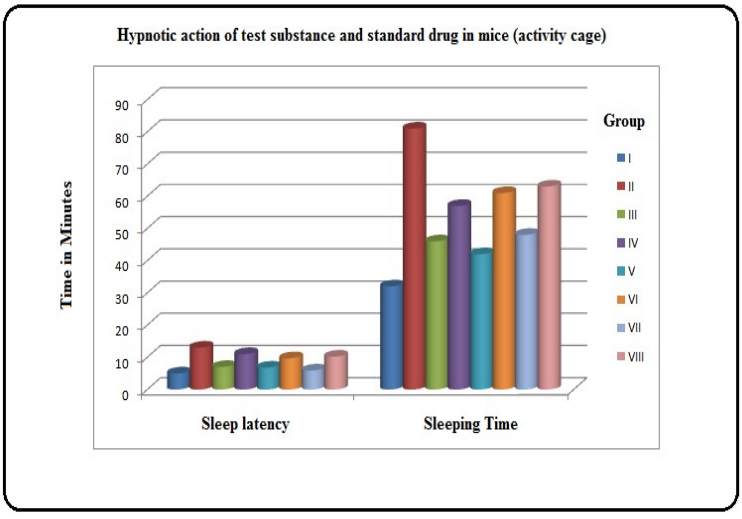

Diagram 4: Hypnotic action in Mice

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