International Journal of Engineering Technology Science and Research
IJETSR
www.ijetsr.com
ISSN 2394 – 3386
Volume 4, Issue 5
May 2017

Effect of Stress on the Hippocampal Region of Brain and use of Centella Asiaticaas a Neuroprotector

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ABSTRACT

In the modern therapeutic system most of the drugs have severe side effects. To overcome this shortcoming use of Ayurvedic drugs are been promoted. We have vast wealth of Ayurvedic, Unani and Siddha systems of medicines, recommending several plants or their extracts having curative or preventive effects against several deformities. Now a days several plants are used as diuretic, sedatives, anti-tumor, anti-ulcer, anti-stress, skin-modifiers, appetite-increaser, etc. In present study Centellaasiatica, which is commonly known as *"Mandukaparni"* has been used as neuro-protector against the neural degeneration with special reference to Hippocampus.The role of hippocampus in memory, learning, discrimination and behavioral activities has now been established. This hippocampal region has found to be directly effected by stress. The concept of stress in biology is used with reference to factors that disrupts normal physiological or psychological function.The use of Centellaasiatica as a neuro-protector has opened a new field to control the adverse effect of stress on the hippocampal region of brain. Improvement has been shown by Centellaasiaticain the hippocampal region of brainfor enhancing memory and intelligence.

1. INTRODUCTION

Brain is made of three main parts: forebrain, midbrain, and the hindbrain. The forebrain consists of the cerebrum, thalamus, and hypothalamus (part of the limbic system). The Hippocampus is a very large area in the limbic; with an overall curving thus was named as "Hippocampus". It is the part of brain known to carry out several fundamental processes that transform information, abstraction, partitioning and recombination, that is formation of conjunctive association between events (Gluck and Myer, 1993). Hippocampus also plays a primary role in identifying a situation as stressful.

In recent years, some of the cellular and molecular mechanism which are involved in learning and memory have been extensively investigated in Hippocampus.

The primary area of the brain that deals with stress is its limbic system. Because of its enormous influence on emotions and memory, the limbic system is often referred to as the "emotional brain."Stress" may be defined as the pattern of bodily responses to any challenge as an event, which precipitates various body changes or as the complex interaction that occur between stimuli, target organism and adaptive responses. Situations, which require adjustment, can be stressful.

Cohen et al (1993) investigated three aspects of psychological stress viz., negative effect, perceived stress and stressor exposure. These three aspects of psychological stress can be seen as providing information about the extent to which an individual feels equipped to handle the stressor to which s(he) is exposed, and also provide information about stress perception and stressor exposure. Majority of previous studies suggest that it is the stress perception, and not stressor exposure, which predict negative health symptoms (Matthews et al, 1996).

In the modern therapeutic system most of the drugs have severe side effects. To overcome this shortcoming use of Ayurvedic drugs are been promoted. We have vast wealth of Ayurvedic, Unani and Siddha systems of medicines, recommending several plants or their extracts having curative or preventive effects against several deformities. Now a day several plants are used as diuretic, sedatives, anti-tumor, anti-ulcer, anti-stress, skin-modifiers, appetite-increaser, etc.

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Branch of Ayurveda known as "*MedhyaRasayan*" constitute several herbal drugs like *Brahmi*, *Shankhpushpi*, *Jatamasi*, *Mandukaparni*, etc. which are used as brain rejuvenators. Extracts of these plants have their affect directly on the brain, improving the short-term and long-term memory.

In present study Centellaasiatica has been used as neuro-protector, which is commonly known as *"Mandukaparni"*. It is a creeper found round the year prominently in the rainy season and in areas with plenty of water. The flowers are small, pink colored and its leaves are slightly thick having pungent smell. The leaves of this herb contain vehlerin, which gets evaporated in sunlight; therefore, it should always be dried in shady places. After drying, the leaves loose their taste and smell.

We have planned to study the effects of stress after its chronic exposure and if Centellaasaticacan produce preventive and repairable effects in the various sub-regions of Hippocampus together with any, observed changes in behaviour of animal after intake of drug under experimental conditions and protocol.

2. MATERIAL AND METHOD

2.1 Experimental Design

a) Control Group

Cages with rats were kept in isolated room and locked for 24 hours to avoid the stressful situation due to handling. Body weight, food intake and water intake of entire group was recorded both prior to the start of an experiment (7 days) and daily during the experiment period (30 days).

b) Stress Protocol

Immobilization stress was given to the rats. Rats were kept individually in tight and ventilated plastic boxes for 2 hours/day for number of days as per protocol. Rats were fitted so tightly that they were unable to move in the box and hence got stressed.

c) Drug Protocol

The marked dose of drug (400mg/kg of body weight) was administered to the animal orally with the help of pearl pointed syringe. The animal was hold vertically upside with mouth placed upside and drug was pushed to the body through the gullet. In experimental group (D) drug was always administered after stress and alcohol protocol.

2.2 Behavioural Studies

For studying the behavior of albino rats two different parameters were taken into consideration:

I) Open field behavior and

II) Reproductive behavior

I) Open field behavior included:

a) Grooming
b) Sniffing
c) Feeding behavior
   i) Body weight
   ii) Food intake
   iii) Water intake
d) Fighting behavior
e) Spatial task

II) Reproductive behavior included:

a) Sexual behavior
b) Maternal behavior

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3. OBSERVATION

3.1. Behavioural Studies

I) Open field behavior

(a) Grooming Behavior

Effect of Stress and Drug only on Grooming time / min

	Control	Stress	Stress + Alcohol + Drug	Drug only
Initial	5	8	12	7
Ist Week	5	8	11	6
IInd Week	6	7	10	6
IIIrd Week	5	7	9	6
IVth Week	6	7	9	6

Under condition of immobilization stress for the period of two hours by conceiving the animal in a ventilated plastic bottle the time of grooming was observed to increase by almost two folds as compared to control ones. It was observed that as soon as the animals were released they open their arms and tired to groom the body fur. This activity lasted for mean grooming period of almost 8 minutes as compared to 5 minutes under control condition during first week. In the subsequent weeks it was observed that the grooming period was almost the same as the control one and altogether there was no change in the behavior of animal to groom itself.

(b) Sniffing Behavior

Rats were left to move in an area demarked by thermocol sheets and were observed from above silently. Following observations about sniffing behavior were gathered:

i. Male with male/Female with female

When members of same sex belonging to different cages were released in an open area, it was observed that sniffing was shown by each rat at the genitalia of other moving in a 'go-round' manner, trailing each other in a stereotyped way. Ultimately this causes aggression and then fighting leading to jumping and biting each other.

ii. Rat with new object

The sniffing activity was observed when food and new drinking water bottle was placed in the cage. Even when cotton bundle was placed in the cage rats sniffed it several time and then showed exploratory behavior.

iii. Female/Male

Lordosis was observed by male for the female introduced in the marked area, whereby it sniffs the trail mark of the female and followed it. Finally tracing the female and sniffs the genital of female and if female is not estrus male attempts coitus .

(C) Feeding Behaviour

i)Table: I Effect of Immobilization Stress on Body Weight (gms)

Groups		Initial	1st week	2nd week	3rd week	4th week
Control group	N =	10	10	10	10	10
	Mean	110.21	110.20	111.19	111.25	112.13
	S.D.	1.16	1.19	1.19	1.27	1.45
Experimental Stress	N	10	10	10	10	10
	Mean	111.20	109.23	108.74	106.18	105.11
	S.D.	1.22	1.12	1.11	1.10	1.12
	t-value	1.855	1.877	5.944***	9.534***	12.108***

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Under the condition of immobilization stress in the albino rat it was observed that body weight of animal as compared to control ones. Decline was found non significant in the first week, significant in the second week while highly significant decline was observed up to the fourth week.

ii) table 2: Effect of Immobilization Stress on Food Intake (gms/kg body weight)

Groups		Initial	1st week	2nd week	3rd week	4th week
Control group	N =	10	10	10	10	10
	Mean	324.44	322.84	323.61	324.24	325.13
	S.D.	1.23	1.30	1.21	1.34	1.26
Experimental Stress	N	10	10	10	10	10
	Mean	325.14	321.41	317.53	310.49	304.57
	S.D.	1.12	1.20	1.32	1.08	1.58
	t-value	1.327	2.554*	10.760***	25.279***	32.223***

Under the condition of immobilization stress on albino rat it was observed that as compared to the control group a non significant decline was observed in the first week while a highly significant decline was observed in the subsequent week till the end of the experiment.

iii) Table 3: Effect of Immobilization Stress on Water Intake (ml/kg body weight)

Groups		Initial	1st week	2nd week	3rd week	4th week
Control group	N =	10	10	10	10	10
	Mean	201.35	204.23	208.53	211.59	213.29
	S.D.	1.14	1.18	1.16	1.52	1.22
Experimental Stress	N	10	10	10	10	10
	Mean	202.32	211.19	219.24	228.25	235.15
	S.D.	1.28	1.47	1.49	1.50	1.48
	t-value	1.782	11.670***	17.962***	24.748***	35.991***

Under the condition of immobilization stress a significant increase in water intake was observed as compared to the control group animals. This increase was found to be highly significant from the first to fourth week.

d) Fighting Behavior

Fighting behavior was observed, when a stranger especially a male was introduced in a cage. Typical assault begins among rats, and strangers are attacked, but any sort of retaliation was not observed. It was noticed that if a stout host male approaches an intruder, his teeth chatter and hair gets raised, as the defendant defecates and urinates in the cage. Defendant shows gestures of surrender like turning his flank, adopting characteristic threat posture like extending his legs and arching the back. The intruder rat was found moving round the opponent with mincing steps. The resident then leaps straight at the weaker intruder with rapid movement of his forelimb nipping any of the body parts like limb, ear or tail. The intruder is often left stretched out simply breathing rapidly and irregularly with biting marks on bedraggled and staring fur, but activity was soon found normal with larger host male involved in some other activity leaving intruder in one corner. The fighting is more vigorous and frequent in presence of a female.

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i) Stress

No significant effect was observed when immobilization stress was given to either of the intruder or to the host animal of the cage.

Effect of Stress and Drug only on Spatial Task Tests

- **Spatial Task**

	Control	Stress	Stress + Drug	Drug only
Initial	6	6	7	6
Ist Week	6	6	6	6
IInd Week	6	5	5	6
IIIrd Week	7	5	4	6
IVth Week	7	4	5	7

The immobilization stress given for two hours reduced the attempts of successful trials in treated group. While spatial task showed a decline in performance by alcohol treated group severely. The decline becomes more pronounced in the animals treated with stress and alcohol protocol. Drug alone when given to the albino rats caused an initial decrease in successful trials with a slow rise to reach a appreciable success in crossing the bar. Direct effect of drug administration was observed with improved memory and thus an improved spatial task performance as compared to impaired behavior of animal under synergistic effect of stress and alcohol.

II) Reproductive Behavior Included:

a) Sexual Behavior

Such behavior is species specific. This includes filial behavior of rats. Sexual behavior includes parental behavior as well as their patterns of submission and attack. These are regulated by the signals. When an adult male rat encounters a mature female, he sniffs genitalia, if she is not in estrus male attempts coitus.

If the female is receptive she herself approaches male gently touching his flank with her nose. In normal condition the male generally takes the initiative, and follows the odorous secretion to reach female counterpart. Such a behavior is also observed when males are left in a new cage. Whenever a single female is introduced in the cage the behaviour of male rats gets changed. They fight for sexual satiation and unsuccessful ones get frustrated. Evidently, sexual excitement without consummation lowers the threshold for territorial fighting among males. Resident males attack new comers more vigorously in the presence of females.

Stress

After one day of immobilization stress for 2 hrs there was no significant changes observed in sexual behavior, but as the time passed the sexual desire was observed to decrease. This was prominent after 15 days post treatment. After 30 days they show least sexual activity. Animals when released from restraint condition were found dull and confined to corners of cages. The members of opposite sex had no interest in each other. This situation was only observed after one-month period.

b) Maternal Behavior

Control

Animals when expecting, prepared nest as a part of parental care. In rats, it is seen that females gathers all the required material for nest making (Plate). Nest making is a natural instinct, since in the cage there is no such material available for nest making animal was found moving impatiently round the cage as if trying to make the nest for her satisfaction. As soon as cotton was made available, animal made a paddy gathering and sat on it to give birth to young ones. When the young were born they are licked and assembled

in-group in the nest mostly one above the other. If they are not licked they do not develop reflexes and soon die. It was observed that litter could not survive without mother. When the young fall or wander, crawling out of the nest they are retrieved by mother. The young themselves make high-pitched sounds, when they needed food. A female employs several senses in sensing their young ones like appearance and odor. It was noticed that crawling evidently interferes breeding females with litters, may be pestered by male, although ordinarily a parturient female drives away intruders from her nest merely by making sound and perhaps a snapping movement of her head

Stress

The stressed mother as previously quoted had low rate of reproduction also found to be less active in retrieving the young one within the nest and was found less aggressive towards intruders coming towards nest. Stress for 2 hrs. daily given for 30days did affect marked behavior to a large extent besides low rate of conceivement in stressed females.

4. RESULT AND DISCUSSION

The role of hippocampus in memory, learning, discrimination and behavioral activities has now been established.

Stress is a condition or stimulus that threatens an organism's survival (Chrousous and Gold). Stress can be beneficial or detrimental to the survival of an organism, leading to behavioral or physiological changes (Herman and Cullinan,).

This stress system has a close integration with other central nervous system and the elements involved in the regulation of behavior and emotion in addition to axis responsible for growth, immunity and reproduction (Abraham and Gogate, and Baum et al). The brain sub-regions has been found to be directly affected by stress expressed in the form of varying degree of cellular degeneration resulting into impaired memory. Stress, thus has been found to be directly involved in causing Hippocampal degeneration.

The result of present study demonstrated that one month of immobilization stress caused significant degeneration in Hippocampal cells which were seen in form of pyknotic, condensed and shrunken cells lacking cytoplasm.

The consistency of our result was not found with the results given by Uno, who did not reported any loss of pyramidal cells in Hippocampal region of female monkey. Another, contrary finding was given by Harding et al where it was reported that changes observed in rodent model of alcoholism were not found parallel to those observed in the humans.

Fuch and Flugg confirmed degeneration of Hippocampal sub-region due to chronic stress causing dendritic atrophy of Hippocampal pyramidal neurons due to elevated level of Glucocorticoid

Another probable reason, which explains Hippocampal damage due to prolonged immobilization stress, may be due to the fact that stress regulates neurotrophic factor expression in the brain. Stress has been found to decrease brain derived neurotrophic factor (BDNF), mRNA in Hippocampus leading to stress induced damage (Smith). Nerve growth factors (NGF) and neurotrophin 3 (NT3) get increased by stress and Glucocorticoid perhaps as a compensatory response to stress induces damage.

Neurotrophic factors being neuro-protective in nature may also be effective in reversing Glucocorticoid-induced damage to Hippocampus. These factors plays very important role in protecting the brain from variety of traumatic insult. The advance research in the field of neuroscience suggest that oxidative stress may also play an important role in neuron loss in neuronal degenerative diseases such as Parkinson and Alzheimer's disease. Oxidative stress is a cyto-pathological condition caused due to mismatch between free radical production and ability of cell to define against them (Simonian).

Neural degeneration caused due to stress may be checked with herbal drug categorized under MedhyaRasayana. In present work Centellaasiatica has been taken as neuro-protector.

The use of Centellaasiatica as a neuro-protector has opened a new field to control the adverse effect of alcohol and stress on the brain. Improvement as shown by Centellaasiaticafor enhancing memory and intelligence needs further studies to control and recover neural degeneration caused by stress, alcohol intake and other day to day factors.

5. ACKNOWLEDGEMENT: The authors are thankful to the Head of the Department of Zoology, University of Rajasthan, Jaipur and Department of Zoology, Govt. R.R.(PG) Autonomous College, Alwar (Raj.) for providing laboratory facilities.

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