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Histological Study of Action of Alcohol on Hippocampal Region of Brain and Use of "Mandukaparni" as a Brain Rejuvenator

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ABSTRACT

"Hippocampus" is the part of brain known to carry out functions like learning, transforming information, discrimination,etc. The activity in the Hippocampus is disrupted by alcohol intake, interacting directly with Hippocampal neurons. In present study Centella asiatica has been used as neuro-protector, which is commonly known as "Mandukaparni". "Mandukaparni" is sour and sharp in taste, easily digestible, having cold effect, nutritive and fever curer. In the present study special emphasis has been laid to see the histo-pathology of Hippocampal sub-regions, created due alcohol intake on albino rats and efforts have been made to correlate the exhibited neural degenerations in CA1, CA2, CA3, CA4 and Dg sub-regions of Hippocampus with behavioral changes. Here effectiveness of drug has been shown on learning and memory and it is classified as Medhya rasayan.Our results reveal Centella asiatica as neuro-protective agent; it's use with alcohol showed least degeneration in different sub-regions of Hippocampus.

1. INTRODUCTION

Brain is made of three main parts: forebrain, midbrain, and the hindbrain. Limbic system is situated in the forebrain. The limbic system is the term for a collection of brain structures, located in the vicinity of the thalamus and hypothalamus, which together participate in the neural processing of emotional behavior. The major structures in this system are the mamillary body, the septum (or septal area), the Hippocampus, the amygdala and lingulate cortex.

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 functions like learning, transforming information, discrimination,etc. It is involved in navigation, recall of long term allocentric spatial information and context dependent episodic memory but not visual pattern matching. Studies also shows the importance of Hippocampus in spatial memory, the ability to remember where an animal has been and where critical events have occurred (Morris et al; O' Keefe and Conway).

A cross-section taken perpendicular to the long axis (septal-temporal) will reveal the internal structure as two interlocking "Cs", one reversed in relation to the other, each with its own principal cell layer. One "C" makes up Ammon's Horn or Cornu Ammonis (CA1 -CA3), also known as the "Hippocampus proper". The principle cell layer of Ammon's Horn is the stratum pyramidale, or the pyramidal cell layer. The other "C" is made up of the Dentate Gyrus, of which the stratum granulosum, or granule cell layer is the principal cell layer. Although the Dentate Gyrus is commonly included as part of the Hippocampus, it is cyto-architectonically distinct from the Hippocampus proper (the architecture, or structure of the cells are different. However, sometimes the Hilus of the Dentate Gyrus, the area inside the "C" created by the granule cells is referred to as CA4. The pyramidal cell layer of the CA l-CA3 regions begins to breakdown as a tightly packed cell layer and becomes more sparse in this region, such that early neuro-anatomists did not distinguish between these areas. Subsequently, and partially because the Dentate Gyrus is not truly Hippocampus, terms like "Hippocampal formation" are used to discuss Ammon's Horn and the Dentate Gyrus together. The intrinsic connections between the principle cell layers of the Dentate Gyrus and CA regions of the Hippocampus are very clear.

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Alcohol

Alcohols are all toxic to humans. The effects of alcohol on the brain can occur by both direct and indirect means; indirectly through alcohol-induced deficiencies in nutrition, liver disease, and through alterations of the function of other bodily systems (e.g. immune, hormonal), which produce substances that end up in the blood and get transported to the brain.

The brain is the organ most sensitive to alcohol. It also receives less oxygen when alcohol is present. For individuals who drink to excess, the clinical picture is highly unfavorable (Maher). Except for about 5-10% that is eliminated through breath, urine and perspiration, alcohol that is taken in, has to be assimilated by the body itself.

Alcohol besides affecting other body parts has most detrimental effect on brain. It significantly lowers performance on cognitive ability tasks (Pickworth et al) such as problem solving. More complex the task, the more is the organic impairment, including brain shrinkage, observed in a high proportion of alcoholics (Errico et aL), especially among binge drinkers, people who abuse alcohol following periods of sobriety (Hunt). Studies show that most problem drinkers are men, with about five times the frequency of women (Helzer et al).

Alcohol has complex and seemingly contradictory effects on the brain. At lower level, alcohol stimulate certain brain cells and activates the brain's "pleasure areas", which release opium like endogenous opioids that are stored in body (Van Ree).

At higher levels alcohol depresses brain functioning, inhibiting one of the brain's excitatory neurotransmitters, glutamate, which in turn slows down activity in parts of the brain. Inhibition of glutamate in the brain impairs the organism ability to learn and affects the higher brain centers, impairing judgment and other rational process and lowering self-control. As behavioral restraints decline, a drinker may indulge in the satisfaction of impulses ordinarily held in check. Some degree of motor dis-coordination soon becomes apparent and the drinker's discrimination and perception of cold, pain and other discomforts are dulled.

The activity in the Hippocampus is disrupted by alcohol intake, interacting directly with Hippocampal neurons.

Normally it is observed that consumption of alcohol is a result of physical or mental stress and alcohol is taken as relaxant. Efforts have been made in present study to prove detrimental effect of alcohol in different sub-regions of Hippocampus.

Drug

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 in Ayurvedic system several plants are used to overcome the side effects in modern therapeutic system.

Branch of Ayurveda known as "Medhya Rasayan" 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 Centella asiatica has been used as neuro-protector, which is commonly known as "Mandukaparni".

"Mandukaparni" is sour and sharp in taste, easily digestible, having cold effect, nutritive and fever curer. It is also useful in bronchitis, inflammation and asthma. It improves memory, diet and voice. It also improves the blood vessels inside the skin so; it is very helpful in diseases related to skin and urinary system. Indian practitioners are using "Mandukaparni" to cure various skin diseases.

In this study we have planned to see the effect of alcohol after its chronic exposure and preventive and repairable effects of Centella asatica in the various sub-regions of Hippocampus and to observe changes in behaviour of animal after intake of drug under experimental conditions and protocol.

The present study has been taken to project and take up further research for establishing neuro-protective nature of "Mandukparni" against the neural degeneration with special reference to Hippocampus. Aspect was chosen keeping in mind the fact that, a vast potential of medicinal plants are lying untapped having very positive clinical results with least side effects and we still lack a serious effort to promote Medhaya rasayana as a safe and effective alternative to existing synthetically manufactured organic drugs for treating the neural degeneration.

In the present study special emphasis has been laid to see the histo-pathology of Hippocampal sub-regions, created due alcohol intake, immobilization stress and their synergistic effects on albino rats. Efforts have been made to correlate the exhibited neural degenerations in CA1, CA2, CA3, CA4 and Dg sub-regions of Hippocampus with behavioral changes.

2. MATERIAL AND METHOD

i) 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). The animals were decapitated immediately after taking out from cage and brain was dissected, trimmed and fixed in 10 % neural formaldehyde at 4ºC for 16 hours.

ii) Alcohol Protocol:

Pearl pointed syringe was used for administration of marked dose (3gm/Kg of body weight) of alcohol to the animal (West et al, 1985). To give alcohol, albino rats were hold vertically with mouth placed upside and then alcohol is pushed through gullet into the body with the help of syringe.

iii) 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, drug was always administered after alcohol protocol.

Histological Study

The rats were sacrificed by decapitation after cervical dislocation without any anesthesia, brain was dissected out immediately, after washing with distilled water, tissues were fixed in 10 % natural chilled formaline or bouin's fixative for 16 hr. at 4º C. Tissue was rinsed in distilled water after fixation is over. They were dehydrated in alcohol series cleared in xylene and embedded in paraffin wax. Sections of 10µm thickness were cut on rotatory microtome. Coronal sections containing Hippocampal sub regions (Bregma – 2.3 mm to 4.3 mm) were used for histological protocol. Alternate sections were picked upon slide to make 3-4 series of sections required for various staining protocols.

Sections were deparaffined in Xylene and dehydrated in descending series of alcohol, treated with 0.5 % lithium carbonate and stain in 1% crystal violet rinsed in distilled water dehydrated in n butyl alcohol, cleared and mounted. Alternate series of sections was stained with crystal violet and pyronin Y for demonstration of nissyl substance.

Alcohol

To study neural degeneration in Hippocampal sub regions in alcohol treated rats:

i. 40 animals were taken for study.
ii. 10 animals which were subjected to alcohol protocol for one day were decapitated and subjected to normal histological treatment using crystal violet stain.
iii. Another set of ten animals were administered alcohol for15 days and decapitated, sections of Hippocampus were cut in cryostat and subjected to crystal violet stain.

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iv. Ten animals (housed in 3 cages) were treated in alcohol protocol for 30 days and were finally decapitated for histological sectioning for studying Hippocampal sub region using crystal violet stain.

v. A set of ten animals kept for control study, were decapitated on 30th day for histology.

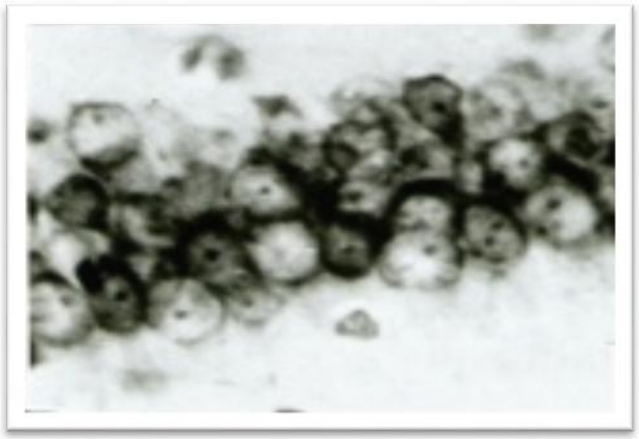
3. OBSERVATION

Histology

After subjecting the animals to alcohol they were decapitated and histological slides of their brains were prepared to expose the CA1, CA2, CA3, CA4 & DG region of their hippocampus. Neuron cell bodies of these sub-regions were observed under magnification (X40) of microscope. Identification of Hippocampal sub-regions was based on "Rat Brain" in stereotaxic coordinates atlas.

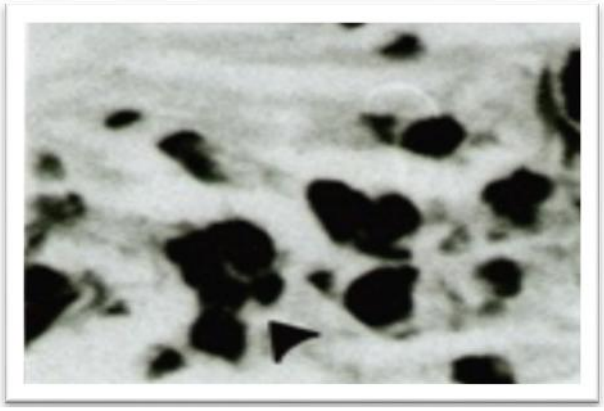
Following changes were observed in different sub-regions of hippocampus.

In control condition, almost all the cells of hippocampal sub-region were found to be normal with compact arrangement of cells in 4-5 layers . All the pyramidal cells appeared normally with rounded shape, clear nucleus and clear cytoplasm. The cells exhibited normal staining with crystal violet (Slide 1).



Slide 1

No significant change was observed after 1 and 15 days of chronic alcohol administration in different sub regions of Hippocampus. Severe degeneration was observed after 30 days of alcohol administration in the form of irregular, darker and pyknotic cells. Irregular perikarya was observed with atrophic dendrite branches. Arrangement of cells was also very loose as compared with the cells of control condition (Slide 2).



Slide 2

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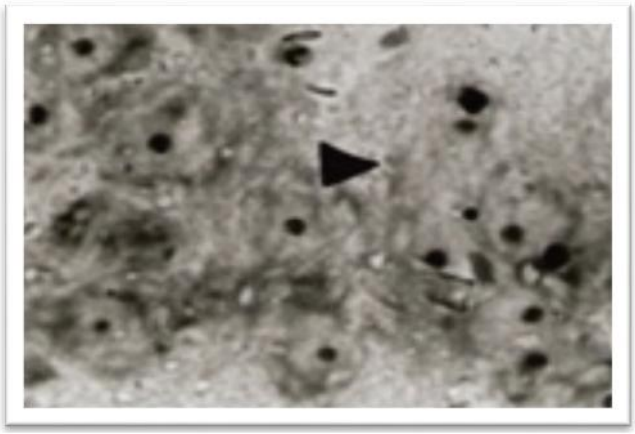
When drug (Centella asiatica) was administered to the animal after alcohol then no damage was observed. Rounded cells with prominent nuclei were observed and they were compactly arranged. Sections were comparable to the control ones. Negligible sign of neural degeneration was observed in hippocampal region in this experimental condition exhibiting neuro-protective nature of drug (Slide 3).



Slide 3

4. RESULT AND DISCUSSION

Alcoholism is found to be the third largest killer disease in the World, besides cancer and heart attack. Alcoholics may be defined as those excessive drinkers whose dependence on alcohol has attain such a degree that it shows a noticeable mental disturbance of interference with their physical and mental health.

However, alcohol related neuronal loss has been found in specific region of the cerebral cortex (superior frontal association cortex) and hypothalamus (supra optic and paraventrical nuclei). But no change has been reported in basal ganglia nucleus basalis or serotonergic raphe nuclei. The functional changes and cognitive deficits in uncomplicated alcoholics have been documented due to dendritic and synaptic changes together with changes in reception and transmission that precedes the more severe structural neuronal changes. Neuronal loss in hypothalamus and cerebellum causes pathological changes that have been found to correlate with alcohol intake (Harper and West et al).

More vulnerability of mature neurons to ethanol induced hypoxic episodes may be accounted for neural degeneration cause by alcohol. The probable reason for this may be high dependence on oxidative phosphorylation and use of ATP. Mature cells may also be more sensitive to ethanol-induced disruption of intracellular Ca++ (Michaelis and Michaelis).

Newlin, in 1981 evaluated effect of ethanol on synaptic transmission in rat Hippocampus, since the action of psychoactive drug effect the neuronal activity. Indeed the systemic application of ethanol at doses associated with behavioral intoxication increase the excitatory and inhibitory responses of pyramidal cell to stimulation of different pathway. The systemic does of ethanol selectively potentiates excitatory responses to acetylcholine(Ach) and inhibitory responses to somatostanin-14(SS-14)but do not affect responses to other neurotransmitters in Hippocampus.

Therefore, in the recent times, there has been a massive hunt for all such herbal formulations, which are effective against neural disorders. Centella asiatica has been used as neuro-protector against synergistic action of alcohol and drug with the same intension. The drug was found to be effective in preventing further neural degeneration and thus controlling damage to Hippocampal cells caused by action of alcohol. Hippocampal sections of experimental animals after treatment with drug shows least neural degeneration in CA1, CA2, CA3, CA4 and Dg region thus exhibiting the neuro-protective nature of drug.

Extract obtained by Centella asiatica is commonly used in the Ayurvedic system of medicine to treat various diseases. Our study gained support from the work of (Babu, Kuttan and Padikkala), they reported that crude extract and partially purified fractions of Centella asiatica has anti-tumer effect in mice.

Centella asiatica shows improvement in the central nervous system, as its asiaticoside derivatives were found to inhibit or reduce H2O2 induced cell death and lower intracellular free radical concentration, protecting against the effect of ß amyloid neurotoxicity (Mook Junk et al,). Our study for using Centella asiatica as neuroprotective drug is supported by the work done by Leung and Foster. They studied the assessment of turnover of biogenic amines (nor-epinephrine, dopamine and serotonin) and showed significant reductions of these amines and their metabolites in the brain following administration of fresh juice. The decrease in amine levels was correlated to improved learning and memory in rats.

Leung and Foster, observed that a water-soluble fraction of Centella asiatica was found to have an anxiolytic effect in animals comparable to diazepam. The extract of Centella asiatica was found to increase brain GABA levels (Chatterjee at al,).

Heinerman, observed that *Mandukaparni* is exceptionally high in ß Complex vitamins, especially B1, B2 and B6 all of which are essential for the correct function of the nervous system.

Our observations got due support with results of (Jaiswal & Bhattacharya), where effectiveness of drug has been shown on learning and memory. Categorizing it as a nootropic agent and classifying it as *Medhya rasayan.*

Our results reveal Centella asiatica as neuro-protective agent; it's use with alcohol showed least degeneration in different sub-regions of Hippocampus.

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