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Effect of Fructus Psoraleae on Wistar Albino Rats' Ethanol-Induced Neurodegeneration of the Hippocampus

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Abstract:
Alcohol may harm the brain in the same ways it does other organs. It causes brain atrophy and cognitive impairment, either permanent or temporary. Thus, alcoholism has been a major challenge for humankind, yet it remains ingested as one of the most popular psychoactive drugs in the globe. It has been calculated that alcohol contributes to 3.8% of all fatalities worldwide and 4.6% of global DALYs. Due to gender-based disparities in consumption and drinking patterns, men in India suffer greater alcohol-related damage than women. The effects of fructus psoraleae on ethanol-induced hippocampal neurotoxicity in wistar albino rats are investigated here.

1. Introduction

Ethanol impairs cognitive processes including learning and memory, making it one of its primary effects. In particular, it causes problems with computation, short-term memory, and concentration. Ethanol's known impact on cognitive abilities prompts the issue of whether anatomical circuits are temporarily or permanently disrupted.[1-2]

In terms of neuropathology, alcohol abuse causes damage to the medial diencephalic area, which includes the hippocampus formation, and other subcortical centers. There has been a lot of progress in the study of neuroscience, and it is now understood that the hippocampus formation is crucial to the processes of learning and memory. The limbic system's hippocampus takes the form of a seahorse. [3-5]

Due to its subdivision into subfields CA1, CA2, CA3, and CA4, the hippocampus proper is also known as Cornu Ammonis. Similar to the neocortex, the hippocampus proper and dentate gyrus are structurally composed of three distinct layers: an outside molecular layer, a middle neuronal layer, and a deep polymorphic layer. The dentate gyrus' intermediate stratum is made up of granule cells, whereas the hippocampus proper's intermediate stratum is made up of pyramidal cells . The hippocampus is a brain region thought to play a crucial role in declarative memory. [6-7]

Declarative memory may be broken down into two subtypes: semantic memory and episodic memory. In contrast to episodic memories, which store concrete instances like going to class, semantic memories retain more abstract, factual information like the sorts of food. Both animals have had their hippocampi shown to play an essential role in declarative memory.[8-9]

The hippocampus's size has been hypothesized to be proportional to one's retentive skills. The hippocampal region is thought to be especially susceptible to oxidative stress because it generates oxygen free radicals at a rapid pace yet has a weak antioxidative

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defense system. Hippocampal antioxidant defenses are depleted due to the production of cytotoxic acetaldehyde from the oxidation of ethanol, which may be further oxidized to acetate by the acetaldehyde dehydrogenase enzyme.[10]

2. Material and Methods

Animal model

Thirty mature female Wistar albino rats were used in the experiment, all of whom were in good health. Regularly cycling Wistar albino rats (22020g) were purchased from Institute in Lucknow. The rats were housed in clean, sanitary circumstances with access to water and a typical rat pellet diet. For 48 hours previous to the experimental treatment, rats were acclimated to laboratory settings to reduce the effects of any nonspecific stress. Before any tests on animals were conducted, Saveetha University's IAEC gave its permission. Ethical standards and criteria established by CPCSEA were adhered to throughout all procedures and studies.

Preparation of extract

In a small herbal store in Chennai, India, we were able to obtain fresh FP seeds. After being washed, pelleted, and air-dried for 8 hours, it was put in an incubator and heated to 40 degrees for another 2 days to finish drying. Dried FP seeds were ground into a moderately fine powder and then sieved through a No. 44 mesh screen. Methanol was used in a soxhlet system to extract the powdered medication. The extract was concentrated after being filtered. The crude extract of FP was screened for the presence of its components using traditional phytochemical screening procedures.

Experimental design

Thirty adult Wistar albino rats were used in the study, which lasted between two and four months. The rats were allowed to get acclimated to the laboratory setting for a full week before the experiment began. After that, we randomized the rats into five separate groups of six. Two months of age. Except for the first group, which had a sham surgical intervention (SHAM procedure) under anesthetic, all of the rats underwent ovariectomies.

Surgical Removal of the Ovaries

After trimming away the hair with shears and disinfecting the region with 70% alcohol, a single longitudinal skin incision was performed on the dorso-lateral area, at the level of the lower poles of the kidney. The ovary was revealed via a narrow muscle mass slightly below the dorsal muscle mass, and the skin was retracted laterally to one side. Each incision was as short as necessary to facilitate ovary removal. Chromic catgut was used to ligate the uterine arteries and the upper horn of the uterus. Wounds from the ovary removal were sutured closed. There was a strict adherence to aseptic technique all through the process. Procedures were performed throughout the ovulatory periods of the menstrual cycle, as identified by PAP smears of the cervix. This research included five separate groups totaling six female rats.

Control group:

Group I: The dummy control group that did nothing. After the operation, the rats were kept in a completely stress-free environment. Two months had passed since the beginning of the trial when these rats were first housed. The rats were killed after two months.

Experimental groups

Group II: OVX group

Group III: FP orally administered at a dosage of 25 mg/kg once day through gavage for 2 months in patients with OVX.

Group IV: Two-month ethanol-induced OVX rats.

Group V: Two months of FP 25 mg/kg gavage treatment in ethanol-induced OVX rats.

The rats were administered FP and ethanol at this time. Both ethanol and water were stored in a cage where they could be accessed at will.

Fixation by Tran cardiac Perfusion

After the sacrifice, a 4% paraformaldehyde in 0.1 M phosphate buffered saline solution was transcardially perfused to effect fixation. The rats were placed on a tray, and the thoracic cavity was opened by incising the top half of the trunk without causing any damage to the organs within. A cannula was put through the top of the left ventricle after the thoracic cavity had been opened, and it was then maneuvered into the ascending aorta. Once the cannula was in place, it was hooked up to a perfusion pump using 0.1 M phosphate

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buffer saline in order to flush the system and remove any remaining blood. A 500 cc infusion of 4% paraformaldehyde in 0.1M phosphate buffer saline was then gently infused over the course of 1 hour.

For tissue processing

- The use of buffered formalin as a fixative
- Cry sectioning and paraffin embedding materials.

Tissue Collection

The perfused area was then shaved and the skin reflected laterally. The skull was then carefully removed using scissors to reveal the brain underneath the meninges without causing any damage to the brain. Laterally reflected incisions were made in the meninges. The cerebrum was revealed when the brain was carefully raised with blunt forceps. At this point, a computerized balance accurate to within 0.0001 g was used to weigh the brain. The insula, located around 2.6 cm from the frontal pole, was where the brain was severed coronally. The hippocampus, located in the temporal lobe, is found here. When the neocortex of the rat's posterior and temporal regions are removed, the hippocampus, which is formed like a sausage, becomes visible. Part of it was dorsal and located behind the septum, part of it was posterior and curved ventrally and laterally, and the rest was dorsal and located in the temporal lobes of the brain.

The hippocampus proper is what you see protruding from the sausage's dorsal side, while the fascia dentata is tucked up within and lying on its underside. The fimbria was a sizable fiber tract that could be seen towards the lateral aspect of the uncovered hippocampus. The hippocampus was subsequently taken out. Tissue was preserved in 0.1 M phosphate buffer (pH 7.4) with 1.25% (vlv) glutaraldehyde and 1.0% (w/v) paraformaldehyde for a period of time after perfusion. A computerized balance accurate to 0.0001 g was used to weigh the hippocampus. Formalin with a buffer was used to preserve it. Cryosection and paraffin embedding were then performed on the tissue. The hippocampus was preserved by microscopically slicing paraffin blocks at 7–10 m and 20–50 m for cryosection. The hippocampus and cerebral cortex were found at both the cranial and caudal ends of the dissected brain. After being fixed in 4% paraformaldehyde, the tissues were maintained there for at least three days.Reasons for Using Steep Slopes

The hippocampus was preserved in paraffin and then sectioned using a micrometer to a thickness of 7-10 m for paraffin sectioning and up to 50 m for cryosectioning. The purpose of this parafin segment was morphometric investigation to determine the range of maximal diameter, total number, and density of hippocampal neurons. Cryosections of the hippocampus were cut to a thickness of 20-50 m. The expression of ER in these neurons might then be studied using this as a starting point. Sections for immunohistochemistry were obtained at a constant interval of 100 m, once every fifth section.

Neuronal morphometrics in the hippocampus

Using a JENOPTIK Prog Res Capture Pro 2.7 in 20X objective and a Nikon E-600 compound light microscope, the stained slides were dried, cleaned, and viewed. Since more neurons may be seen at once and their outlines are more distinct at 20X magnification, duplicate counts of individual neurons can be avoided. Using Prog Res image processing software, the neuronal diameters were calculated. For this purpose, the hippocampuses of rats were serially sectioned, dyed, and analyzed every fifth section. Thirteen stained slices were counted for each hippocampal tissue sample. Twenty nucleated neurons were selected at random in every fifth region and their diameters were recorded. After taking readings from a set of neurons in one field, we rotated the field by 90 degrees counterclockwise to take readings from the next set of neurons.

3. Results

Histological observations result

Group I

The hippocampus's topography was examined at 40X magnification.

Cresyl violet stains, hematoxylin and eosin, silver stain, and toluidine blue stain were used to examine cells in the CA 3, CA1, and dentate gyrus areas at 40X magnification. There is a layer of tiny neurons in the dentate gyrus, and the hippocampus proper is divided into four regions (CA1-4). The pyramidal layer

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neurons in the CA1/CA3 areas are more susceptible to hypoxia, hence their identification was crucial. The CA3 area begins at the end of the dentate gyrus and extends to the CA3-CA 1 synapse.

The transition from big pyramidal cells in the CA3 area to tiny, densely packed pyramidal cells in the CA1 region marked the boundary between CA1 and CA3. Small, closely packed pyramidal cells formed the CA1 region's three to four rows. The subiculum is a brain region located above the CA1 area. When stained with H and E, pyramidal neurons may be recognized by their big nuclei and the basophilic cytoplasm typical of neurons. The nucleus of several of the neurons were tiny and spherical. Mature mossy cells, also known as oligodendroglial cells, have circular nuclei. Many pyramidal neurons were seen. Nuclei dyed blue with 40X Toluidine blue showed a basophilic morphology. Blue spots could be seen at a distance, whereas chromatin and nucleoli could be made out at a closer look. The nucleus was surrounded by pink, positively charged acidophilic cytoplasm from arginine and lysine.

Cresyl violet stains, hematoxylin and eosin, silver stain, and toluidine blue stain were used to examine cells in the CA3, CA1, and dentate gurys areas. Large numbers of cells were seen with H&E staining. Blue-stained nuclei showed that they were basophilic. Molecular layer and hilus are present in the brains of toluidine-treated rats, as are big pyramidal cells and uniformly distributed dentate cells . SGZ neurons were found to be immature. Neurons were more densely packed in the CA1 and CA3 area than in the DG, as shown by cresyl violet staining of the cytoplasm and, more prominently, the nuclei of cells. The cresyl violet staining was quite dark in the Nissl material. At 100X magnification, the neuronal cell bodies, which are rich in rough endoplasmic reticulum, show as black granules.

At 400X magnification, the pattern of granular and molecular layers inside the hippocampus formation was evident in a Toluidine blue stained slice. The neuropil, a light violet area where axons and dendrites meet, is populated with dendrites that have extended from the dark cell bodies. Characteristic of neurons was the presence of a basophilic cytoplasm in the vast majority of cells. Dentate gyrus containing mossy granule cells. Upper and lower blades of the dentate gyrus are formed by the molecular layer, the granule cell layer, and the hilus, respectively. Granule cells with rounded pale vesicular nuclei and a small number of apoptotic cells with darkly stained pyknotic nuclei were seen in tightly packed layers of granule cells. In the SGZ, we saw immature neurons with oval, black nuclei. Large cells with long processes and very few astrocytes and microglial cells may be seen in the hilus. Pyramidal neurons in the SGZ have dark nuclei and an oval shape. This result corroborated the findings of several writers who found stem cells with astrocytic capabilities in the SGZ.

Group II (100Xand400Xmagnification):

The topography of the hippocampus was examined at 100X and 400 X magnifications. There was no consistency in the pattern of cell dispersion. Small in size, the cells nonetheless had their own nucleus and nucleolus. Cells having constricted or shrunken cytoplasm, pyknotic nuclei, and a high number of vacuolar gaps. Pyknotic nuclei in degenerated cells were easily seen. Cellular cytoplasm and, more importantly, nuclear degeneration was seen, however CA1 hippocampal neuronal damage was less extensive than that in CA2, CA3, and CA4. A small number of mature granule cells and an increasing number of apoptopic cells were seen under a light microscope in slices of the dentate gyrus that had been stained with haematoxylin and eosin and cresyl violet. The cytoplasm of a few cells was faintly coloured. Some hilus cells seemed to have their processes shortened, and their arrangement looked disorganized. Some of the cells seemed to have pyknotic nuclei, which were darkly pigmented. Microglial and astrocyte cell presence was also detected.

Group III

100Xand400XMAGNIFICATION

The topography of the hippocampus was analyzed at 100X and 400 X magnifications. It was seen how the dentate gyrus, CA3, and CA1 were set up. The cell density in CA3 and CA1 was very high in this population. Larger and more uniformly shaped cell bodies were observed. The axon and dendrites originate from this region, which is a globular compartment containing several organelles, including the nucleus. The number of cells with darkly coloured cytoplasm, pyknotic nuclei, and vacuoles was very low. We saw a few cells that had apoptotic bodies.

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There was an accurate display of cellular extension. that communicates with other neurons through projections to their dendrites. Take note of the regularly spaced, closely packed dentate cells and the massive, pyramidal cell.

More granule cells may be found in the granule cell layer. The SGZ included immature neurons. There was an abundance of little cells with spherical nuclei. Dense populations of glial cells and large pyramidal neurons were found in the CA1 and CA3 area. The cytoplasm of pyramidal neurons in the CA3, CA1, and dentate gyrus is darkly stained by cresyl violet stainin. At 100X magnification, the rough endoplasmic reticulum found in neuron cell bodies gives the appearance of black granules in this region. At 400X magnification, the hippocampus formation's granular and molecular layers displayed a distinct pattern. The neuropil, a light violet area, is filled with dendrites that extend from the dark cell bodies and create multiple synaptic connections with axons.

Characteristic of neurons was the presence of a basophilic cytoplasm in these cells. Dense layers of granule cells, which are characterized by their round, pale vesicular nuclei and lack of apoptotic, darkly stained pyknotic nuclei. Subgranular zone neurons were shown to be immature. Triangular in outline, the nuclei of granule dentate cells were often oval in form. Other than things, the hilus is made up of astrocytes and microglial cells, which are big cells with long processes.

Group IV

100Xand400XMAGNIFICATION:

The hippocampus's topography was examined at both 100X and 400X magnification. Apoptotic cells and darkly stained pyknotic nuclei surrounded by astrocytes in the granule cell layer of the dentate gyrus. In several astrocytes and microglial cells were seen. Disarray among the hilar cells was followed by a thinner granular cell layer, fewer mature neurons, and a decrease in the length of neuronal processes. As a result of a decline in big pyramidal cell numbers, cellular density was reduced. Small cells with a misshapen nucleus and nucleolus contributed to an irregular cell distribution pattern. The number of cells with condensed or shrunken cytoplasm, pyknotic nuclei, and vacuoles was much increased. It was easy to see the pyknotic nucleus and degenerated cellular structures. The CA1/CA3 subregion of the hippocampus was much more affected by degenerative alterations than the rest of the hippocampus. Small, spherical, darkly pigmented mossy cells and long, thin nerve fibers were mostly missing.

Group V

Cells distributed normally at 100X and 400 X magnifications. The cells were big, and their nuclei and nucleoli were easily distinguishable. Fewer vacuoles, pyknotic nuclei, and cells with reduced or condensed cytoplasm were observed. Cells in the CA3 area became larger. Glial cells and cell membranes became clearly visible at the increased magnification. H & E staining reveals densely packed neuronal cells in hippocampus layers CA1 and CA3 . More active neurons, moderate infiltrating cell numbers, and a cluster of activated microglia can be seen in all of the sections. Toluidine blue impregnation demonstrates a dense population of CA1 and CA3 neurons. Neurons in the CA1, CA3, and DG regions increased in number. Mossy neurons were seen in the sungranular hilar area. The DG is a sparsely dispersed layer of the hippocampal formation that may be seen in group V's pictorial histoarchitecture. Glial cells, dentate cells, and pyramidal cells all seemed to be in their usual places.

Morph metric analysis

Table1: Brain and Hippocampus Weight (g) following FP Extract or Ethanol Treatment in Rats, Mean ± SEM.

Groups	Group I	Group II	Group III	Group IV	Group V
BrW (g)	1.36±0.03	1.30±0.05	1.48±0.08	1.26±0.05	1.32±0.08
HW (g)	0.077±0.0017	0.063±0.0017	0.098±0.0028	0.061±0.0017	0.072±0.0017

BrW stands for brain weight and HW for hippocampal weight. The ethanol group lost considerably more

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weight (p0.05) than the control group. The ethanol group also had significantly smaller hippocampi compared to the control group (p0.05). The data are shown as a mean ± SEM.

The effects of brain and hippocampal mass on behavior:

In this study, we found that there was a statistically significant (P>0.05)decrease in brain weight (1.26 ± 0.05)g and hippocampal weight (0.061± 0.0017)g in group IV, but no difference in brain weight (P>0.05) between groups I (1.36 ± 0.03)g and V (1.32 ± 0.08)g, and no difference in hippocampal weight (P>0.05) between groups I and V (0.077 ± 0.0017)g. Weight differences between groups were found to be statistically significant (P>0.05) when an ANOVA was performed on brain and hippocampal weights. When weighing the five groups equally, group III had a much heavier brain and hippocampus.

Histomorphometric studies result

Cellular Diameter:

The CA1 and CA3 regions of the hippocampus were analyzed to determine the average cell size. Using this calculation, we were able to determine the diameter of the cell:

Diameter of a cell=Axial ratio × Calibration constant,

Where,

$$\text{Axial ratio} = \frac{\text{Maximum Length + Maximum Breadth}}{2}$$

The ocular micrometer was used to measure the longest and widest cells. Then, we tabulated the findings of measuring the hippocampus cell diameter (d) across all rats (n=6) in a group.

Table 2: Neuronal diameter () in rats treated with FP extractor ethanol, mean ± SEM.

Groups	Group I	Group III	Group IV	Group V
CA1	3.55±0.14	3.33±0.12	2.09±0.14	2.54±0.14
CA2/3	3.15±0.15	3.75±0.25	2.15±0.16	2.45±0.14
CA4	1.90±0.12	1.98±0.14	1.52±0.18	1.88±0.2
Dentate gyrus	3.2±0.18	3.92±0.16	2.68±0.27	2.94±0.29

This table (n=6) was used to determine the average diameter of neurons. The Median Size of a Neuron 2.950.15 in group I, 2.320.19 in group II, 3.250.17 in group III, 2.110.19 in group IV, and 2.450.19 in group V. According to the data presented above, neuron diameter reduced in group IV while under the influence of ethanol. However, it was elevated in group III compared to the other groups. Therefore, this demonstrates that the group's neuron diameter increased thanks to FP.

Hippocampal tissue slices were examined using a microscope with a 40x objective lens. Since the calibration constant varies from microscope to microscope, we utilized the same microscope, objective, and magnification for all of our measurements. Neurons were counted at a magnification of 400X.Pyramidal cells in the granule cell dentate gyrus (GCD), CA1, CA2/3, and CA4 were tallied. From the serial sections created for each group, a randomly chosen portion of cells was used for calculations.

Number of square cells in total:

Table 3: Total number of neurons in rats treated with FP extract or ethanol, mean standard error of the mean.

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Group	Group I	Group II	Group III	Group IV	Group V
CA1	202.9±14.2	172.5±12.2	212.7±10.2	162.5±8.2	197.3±16.2
CA2/3	251.7±5.7	196.9±9.3	241.2±6.8	176.9±8.3	211.2±8.3
CA4	168±9.5	140.8±5.5	160±7.5	110.8±7.5	134.2±6.5
Dentate gyrus	928.6±24.5	689.5±14.5	919.5±15.6	589.5±18.5	710.5±17.2

The above table (n=6) was used to determine the average total number of neurons. Group I had a mean standard deviation of 387.8 ± 13.48; group II, 299.9 ± 10.38; group III, 383.35 ± 10.03; group IV, 259.93 ± 10.63; and group V, 313.3 ± 12.05.

According to the data shown above, group IV saw a smaller reduction in neuron count during ethanol exposure compared to the other groups. However, it was elevated in group III compared to the other experimental groups. Therefore, the results of the experiment confirm that FP increased the overall number of neurons.

Number of cells packed into a given area:

The number of cells in a given volume (in cubic millimeter) was calculated.

Table 4: Neuronal packing density (X103/cubic mm) after FP extract or ethanol therapy in rats mean ± SEM

Group	Group I	Group II	Group III	Group IV	Group V
CA1	141.83±7.52	107.17±3.55	136.33±6.74	97.17±5.55	116.6±7.79
CA2/3	143.63±11.1	113.83±4.25	134.33±7.87	103.83±6.27	120.17±3.07
CA4	118.42±5.62	102.5±7.2	114.1±6.84	92.61±7.2	98.35±8.12
Dentate gyrus	234.16±6.50	190.4±8.58	232.18±5.81	180.22±4.58	198.46±8.65

The aforementioned data (n=6) was used to determine the average neuron packing density. Scores ranged from 159.51 ± 7.69 in group I to 128.48 ± 6.77 in group II to 154.24 ± 6.82 in group III to 118.46 ± 7.15 in group IV to 133.39 ± 6.91 in group V. According to the data shown above, group IV had a reduction in neuron packing density during ethanol exposure compared to the other groups. In contrast to the other experimental groups, however, it rose in group III.

4. Conclusion

The current study's findings show that FP helps stress-induced alcoholics' behavioral activities, which in turn enhances their learning and memory. FP has been shown to be effective in hormonal assays for the regulation of cortisol levels in both stress-induced alcoholics and normal individuals. As neuronal activity in the hippocampus reduces with age and estrogen levels drop throughout menopause, it's possible that these two factors, together with stress and aging, contribute to a loss of mental acuity. Since

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estradiol levels naturally fall with age, FP is especially useful for the elderly and stressed individuals in whom they may be deficient. This research provides convincing evidence that neuronal abundance, density, and size all improved in groups III and V. Therefore, an increase in neuronal quantity suggests that progenital neural stem cells have migrated from the subgranular zone and the ependymal layer of the subventricular zone.

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