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# EFFECT OF *CLITORIA TERNATEA (LINN)* PLANT ROOT EXTRACT ON THE NEURONS OF FRONTAL CORTEX AND DENTATE GYRUS OF YOUNG DIABETIC RATS – A PRELIMINARY INVESTIGATION.

# ABSTRACT

## Aim and objective

To study the effect of herbal alcoholic root extract of Clitoria ternatea on the neurons of frontal cortex and dentate gyrus of young diabetic rats.

## Background

The brain is prone for adverse effects of early childhood diabetes which gradually can lead to diabetic encephalopathy. This problem needs an immediate attention to tackle its advanced adverse effects at the earliest.

## Materials and methods

The diabetes was induced in 22 days (postnatal) wistar rats by giving intraperitoneal injection of Streptozotocin at a dose of 60mg/kg body weight. Daily single dose treatment of 100 mg/kg body weight oral alcoholic root extract of Clitoria ternatea was started and continued for a month. At the end of treatment, the animals were sacrificed and brain tissue was subjected to histopathological studies.

#### Results

The data analysis of frontal cortical neurons was expressed as mean+/-SD (standard deviation) and the differences among the groups were assessed by ANOVA followed by Dunnett test. The number of viable neurons between different experimental groups with p value  $\leq 0.005$  was considered as significant.

#### Conclusion

The preventive herbal therapy with the alcoholic root extract of Clitoria ternatea has shown a significant effect on the morphology of neurons of frontal cortex and dentate gyrus.

Key words Dentate gyrus, Hippocampus, Juvenile diabetes, Streptozotocin

## INTRODUCTION

The neurogenesis starts during the fetal life, but its modifications are continued throughout the individuals' lifespan. The frontal cortex, hippocampal and dentate gyrus areas of the brain are potentially involved in the early childhood learning and memory activities. The cerebral cortex is mainly composed of pyramidal neurons of different sizes along with the supporting neuroglial cells which are part of neocortex <sup>[1]</sup>. The neurological defects in the early childhood diabetes include nerve conduction defects seen in different regions of the body. Which has shown lower velocity of conduction in young diabetic patients <sup>[2]</sup>. The 20% of post mortem studies in young diabetic brain has shown abnormal neurological changes. These observations were indicating the much advanced consequence of early disruption in metabolic equilibrium of young diabetic children <sup>[3]</sup>. The neuropathological changes in the cerebral cortex and hippocampal region have shown volume reduction. It was associated with the memory and learning impairments in the diabetic subjects, which could be due to the impact of hyperglycemia induced deranged metabolic pathways<sup>[4]</sup>. The signs and symptoms of cognitive dysfunctions were identified as early as 1922<sup>[5]</sup>. To assess the structural change, the young diabetics were subjected to Magnetic Resonance Imaging (MRI) studies. They have shown reduced brain parenchymal fractions up to 50%. These changes were associated with shrinkage in the total brain volume, when it was compared with the age matched controls<sup>[6]</sup>. Among number of drugs mentioned to treat the mental disorders

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in Ayurveda, the herb Shankapushpi was selected, which belongs to family Fabaceae. A preliminary experimental study by using fresh flowers of Clitoria ternatea has shown significant hypoglycemic and hypolipidemic effects<sup>[7, 8]</sup>.

#### Materials and methods

Juvenile inbred 22 days old Wistar rats (postnatal) of either sex were procured under the animal ethical committee resolution code IAEC/2/1/2008. And the animals were housed in central animal house which maintained with standard environment. The each experimental animal group was containing 6 rats. And there are 3 different experimental groups were including normal control (NC), diabetic control (DC) and treatment groups (CLT). On the 23<sup>rd</sup> day overnight fasted rats were given intraperitoneal streptozotocin injection at a dose of 60 mg/kg body weight <sup>[9-10]</sup>. On the fifth day rats showing fasting blood sugar (FBS) between 200-400 mg/100 ml were only included in our respective experimental groups <sup>[11]</sup>.

#### **Preparation of extract**

Clitoria ternatea herb was grown by the researcher in the college garden and the plant was authenticated by the scientist and botanist of Indian Council for Medical Research (ICMR). By using the soxhlet extraction procedure it was subjected to defating procedure with petroleum ether was followed by repeated extraction by using absolute alcohol<sup>[12]</sup>.

#### Grouping

The diabetic treatment group was started with extract administration (22days old rats+ 5 days diabetic attention period) immediately and it was continued for next 30 days i. e. 27 days+30 days = 57 days. As a preventive therapy the root extract of Clitoria ternatea was given orally by dissolving in distilled water at a dose of 100 mg/kg body weight of extract. The treatment was stopped at the end of the  $30^{th}$ day. Next day the rats were transcardially perfused and tissue was collected and later it was subjected to brain tissue processing and sections were stained with cresyl violet satin. The viable cells were counted by selecting the random distant sections under microscope by using 40 x objectives <sup>[13]</sup>.

#### Microscopic Observations in experimental group

The frontal cortical and dentate gyrus neurons were showing well appreciated comparable features with other different experimental groups in our study<sup>14</sup>. (Figure 1-6)

#### Statistical analysis

The data analysis of Frontal cortical neurons and dentate neurons were expressed as mean+/-SD (standard deviation), the significant differences among the groups were assessed by ANOVA followed by Dunnett test. P value  $\leq 0.005$  was considered as significant.

#### Results

The number of viable cortical neurons between the control and diabetic groups has shown a significant p value <0.001. Similarly the test between the control v/s treatment group has shown the p value was <0.01. Here student "t" test was used to compare the diabetic and treatment groups, which has shown p value  $p \le 0.05$  (Table.1 and Figure.6).

The number of viable dentate neurons between normal control group and diabetic control groups has shown p value < 0.01. Similarly the test between control v/s treatment group has shown significant p value < 0.01. Student "t" test was used to compare the diabetic and

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treatment groups, which has shown the p value < 0.01 (Table.2 and Figure.2).

#### DISCUSSIONS

The Plant species are the well known companions supporting the human life since the time of evolution. The ancient medical literature shows that there are more than 800 plant species are showing antidiabetic effects<sup>[15]</sup>. Several studies have shown that the phytochemicals from the plant origin have shown encouraging results in the treatment of number of ailments. Which are including cancer, heart disease, diabetes mellitus, blood pressure etc. The World Health Organization (WHO) has recommended the evaluation of antidiabetic drugs from natural origin to substitute the existing inevitable use of modern drugs with its adverse effects<sup>[16]</sup>. The brain is much prone for adverse effects of early childhood diabetes leading to diabetic encephalopathy which is characterized by deteriorating structural and functional changes which can be correlated with the deteriorating results of cognitive function tests <sup>[17]</sup>. The diabetic control group is showing significant changes in the brain by reducing the number of viable neurons. It was evidently found that experimentally induced diabetes produces significant reduction in the number of viable cells in the Dentate Gyrus (DG) and in Cornu Ammonis (CA3) region pyramidal neurons <sup>[18]</sup>. Studies by using the chemical markers in young developing postnatal brain has indicated that there is a considerable modification in the neurons of different areas of the brain before an individual achieve relatively a stable number of neurons in the later part of his life. Postnatal period seems to be showing high discrimination in the number of viable neurons <sup>[19]</sup>. Neurogenesis is a complex process modifying dentate gyrus neurons throughout the life, but this effect decreases with advancing age <sup>[20]</sup>. Probably such findings can be correlated with enhancing number of neurons and increased synaptic contacts associated effective cognitive function in young individuals. Which is an important event accelerates naturally during early hassle free childhood. This observation needs further advanced stepwise experimental evaluation to bring new hope in young diabetic citizens.

#### CONCLUSION

By this preliminary experimental observational study we can tentatively come to a conclusion that the early recognition of juvenile diabetes when tacked with an alternate herbal treatment by using alcoholic root extract of Clitoria ternatea can influence by preventing the degeneration and by bringing some possible reversible changes on degenerated neurons in some selected areas of brain of young diabetic rat model. The outcome of this preliminary observational study could be a base for advanced research in this direction.

#### **Competing interests**

Author declares that he don't have any competing interests and also declares that he has not obtained any grant from any sources for his research.

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NORMAL CONTROL



**Figure- 1. Frontal cortex**. 1, 2, Frontal cortex showing viable neurons with clear cytoplasm. 3.Blood capillary



Figure -2. Dentate gyrus1,2, and 3 viable neurons with clear cytoplasm

## DIABETIC CONTROL GROUP



Figure	-3.	Frontal	Cortex	1&2	
Showing	S	hrunken	neurons	with	
chromatolysis,3- Cell with pyknosis					



**Figure -4. Dentate gyrus**.1-Dentate gyrus showing degenerative changes 2- Shrunken cell 3-Showing air vacuoles.

## TREATMENT GROUP



**Figure - 5. Frontal cortex.** 1-Blood Capillary, 2-Frontal cortex of brain showing wide homogenous cortical area, viable neurons without degenerative changes.3- Viable cells



Figure -6. Dentate gyrus. 1, 2&3 Dentate gyrus showing large number of viable neurons seen in dentate gyrus.

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Groups	Mean	SD
Normal Control	110.58	19.62
Diabetic Control	55.9	16.2
PCLT(Treatment)	68.93	20.05

#### Table No.1, Showing Mean and Standard deviation, Frontal cortical neurons

Used analysis of variance (ANOVA) Over all: P < 0.001 Used Dunnett Test Control with Diabetic: P < 0.01 Control with PCLT: P < 0.01

Groups	Mean	SD
Normal Control	43.68	3.13
Diabetic Control	26.62	3.26
PCLT(Treatment)	24.77	3.76

## Table No.2, Showing Mean and Standard deviation, Dentate Gyrus neurons

Used analysis of variance (ANOVA)

Over all: P < 0.001

#### **Used Dunnett Test**

Control with Diabetic: P < 0.01

Control with treatment: p < 0.001

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# Figure-7 & 8 Showing Mean and Standard deviation, Frontal cortical and Dentate Gyrus neurons

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