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Research Article

EVALUATION OF COGNITIVE ENHANCING ACTIVITY OF *VITIS VINIFERA* LINN. ON ALBINO RATS

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ABSTRACT
In the present study fruits of *Vitis vinifera* Linn. (Vitacae) commonly known as Draksh were selected for preliminary phytochemical investigation and pharmacological screening of cognitive enhancing activity. The shade dried fruits were subjected to successive solvent extraction with 70 % ethanol. The extracts revealed the presence of carbohydrates, proteins, cardiac glycosides, alkaloids and flavonoids when subjected to chemical tests. TLC was run for their confirmation of flavonoids in *Vitis vinifera* and than subjected to isolation by preparative TLC method and analyzed by UV, FTIR and HPTLC. In the pharmacological screening, the alcoholic extract was used for the evaluation of cognitive enhancing activity using elevated plus maze & passive avoidance task method with Mentat as Standard by using parameters of step down and transfer latency. Induction was carried out by MES and scopolamine for 7 days. On 7th day the brain was isolated for evaluation of Acetylcholinesterase enzyme activity. The alcoholic extracts (200mg/kg.B.W) showed significant effect when compare to control, there was significant increase in step down latency and decrease in the transfers latency and also decrease in acetylcholinesterase enzyme activity, which was as effective as that of standard drug. Hence it can by conclude that fruits of *Vitis vinifera* contain favonoid and hence possess significant cognitive enhancing activity.
KEYWORDS: *Vitis vinifera* Linn, Scopolamine, Acetylcholinesterase.

INTRODUCTION

Plant *Vitis vinifera* Linn. (Vitacae) commonly known as Draksh, Dry grape, Wine grape, Angur, Kismish, Drakshai, Kotumuntiri is used as vitiated condition of pitta and kapha, such as burning sensation, dispsia, constipation, amentia, skin disease, asthma and bronchitis. The main chemical constituent present in flower is good source of bioflavonoid (Vitamin P), malic acid dehydroascorbic acid, cholesterol, ergosterol, myricetin and quarcetin. The dried fruits are intellect promoting, cardiotonic, cough, hoarseness and thirst[1, 2], adaptogenic and nootropic activities[3], Hepatoprotective effect[4], antidiabetic and antioxidant activities[5], Antimicrobial Activities[6], Bronchodilatory Activity[7].

Cognitive science is a large field, and covers a wide array of topics on cognition. Amnesia is a profound memory loss which is usually caused either by physical injury to the brain or ingestion of toxic substance which affects the brain. In addition the memory loss can be caused by a traumatic emotional event. It is a condition in which memory is disturbed. Cognitive deficits have long been recognized as severe and consistent neurological disorders associated with numerous psychiatric and neurodegenerative diseases, such as Alzheimer's disease, Senile dementia, Parkinson's disease, Huntington's disease, Korsakoff's syndrome, Down's syndrome, Pick's disease, Creutzfeldt-Jakob disease, Trauma, Chronic insomnia, Epileptic disorder and attention deficit disorders[8].

It has been seen in traditional system that some drugs possess intellect promoting and brain tonic effect in humans. However, fruit extracts *Vitis vinifera* Linn have not been scientifically investigated for the same. Therefore the present study is designed to evaluate the same.

MATERIALS AND METHODS

Plant Material

In the present study, fruits of *Vitis vinifera* were collected from local areas of Belgaum, Karnataka. The fruits were authenticated from botanist Dr. R. S. Goudar, Department of Botany, R. L. S. Institute, Belgaum, Karnataka, India.

After authentication, fruits were subjected to drying at room temperature until they were free from the moisture. Finally the drugs were subjected to size reduction to get coarse powder then the uniform powder was subjected to standardization with different parameters.

Extraction Procedure

The shade dried fruits of *Vitis vinifera* were reduced to fine powder (# 40 size meshes) to obtain a powder of desired particle size. The powder material was subjected to soxhlet extraction with 70% ethanolic. After the effective extraction, the solvent were distilled off, the extract was then concentrated on water bath and subjected to chemical investigation and pharmacological screening for its cognitive enhancing[9, 10]

Chromatographic studies

Thin layer chromatography (TLC) studies were carried out for the presence of different phytoconstituents in the extracts. TLC is a mode of liquid chromatography, in which the extract is applied as a small spot or band at the origin of thin Silica gel GF 254 (activated) layer supported on glass plate. The mobile phase migrates through the stationary phase by capillary action. The mobile phase used for *Vitis vinifera* was n-butanol: acetic acid: water (4: 1: 5).

Isolation of Active Principles

Alcoholic extract of *Vitis vinifera* were subjected to thin layer chromatography to detect the various constituents present in it. The plate was developed in a saturated chamber having desired solvent system. After developing the plate was dried and if the band gives fluorescence then it can be easily scraped. Otherwise a small portion of the band was sprayed with detecting agent by taking care to avoid the exposure of remaining plate to spray reagent. Then the band is scraped by measuring the height of sprayed band.

The scraped band was then suspended in desired solvent and filtered on Whatmann filter paper no.1 and washed several times with same solvent. The filtrates were combined and concentrated and reduced to dryness. This procedure was followed for several scrapings. Then the resulted compound was run with original sample to confirm the isolation[11,12,13].

Characterization of Isolated compounds

Soon after isolation of compound from *Vitis vinifera* were subjected to detailed characterization for the confirmation of probable structure of the compounds from spectral analysis.

U.V. Spectra: The compounds *Vitis vinifera* showed the U.V. absorption maxima (λmax) in nm observed in Schimadzu, Spectrometer model. Spectra are given in.

I.R. Spectrum: The KBr disk of compound was prepared by grinding the sample (0.1 – 2% w/w) with KBr and compressing the whole into a transparent disk, under an infrared lamp (model Shimadzu, FTIR-8000 Spectrometer).

HPTLC: HPTLC of alcoholic extracts of *Vitis vinifera* were performed using CAMAG TLC scanner 3 and LINOMAT-V. The isolated phytoconstituents was characterized by spectral studies[14].

Animals

The Wistar Albino strain rats of either sex weighing 150-200g were procured from NIMHANS, Bangalore. They were housed in a group of six per cage and were maintained under natural day and night cycle at 25±2°C ambient temperature, 45-55% relative humidity. They were allowed to acclimatize one week before the experiment. The rats were allowed with free access to standard pellet and water ad libitum.

The experimental protocol was cleared from the Institutional Animal Ethical Committee, K. L. E. S's College of Pharmacy, Belgaum.

Drugs Used

Mentat – A poly herbal preparation containing around 25 different herbs, and is a proven memory enhancing drug available in market. It was procured from Himalaya Drug Company, Bangalore.

Scopolamine – An antimuscarinic agent for induction of loss of memory.

Extracts Used

The alcoholic extracts of fruits of *Vitis vinifera* were used as the test drugs for the evaluation of memory enhancing activity.

Acute toxicity study

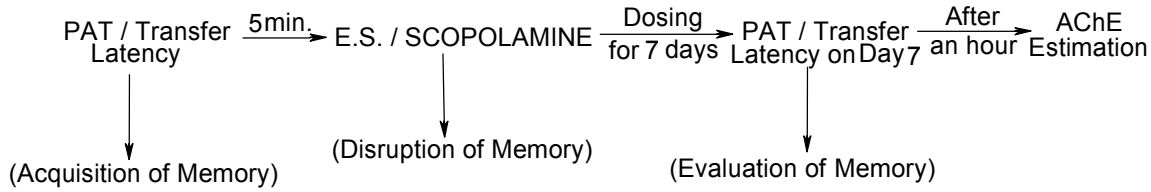
The acute toxicity study was carried out as per the guidelines set by Organisation for Economic Co-operation and Development (OECD) revised draft guidelines 423 B (" Up and Down" method) received from Committee for the Purpose of Control and supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India (See annexure X). The LD_{50} cut-off dose for AEL.AEB, AET $1/10^{th}$ of the LD_{50} dose was taken as therapeutic dose.[15]

Experimental Protocol

The animals were trained on the 0 (zero) day and the acquisition of memory was tested on the day 1, later the animals of Sub Group – 6 & 7 received electroshock of 150mA for 0.2s through a pair of ear electrode from an Electroconvulsiometer and then the animals were dosed with respective drug and kept in their home cage. Similarly, animals of Sub Group – 8 & 9 received scopolamine (0.3mg/kg b.w.) and then were dosed with respective drugs and returned to their home cage. Subgroup 4 serves as control receive only MES and subgroup 5 serve control receive only scopolamine. The electroshock/scopolamine and dosing with drug continued for upto 7 days and on 7^{th} day, the animals were subjected to the retention test 25min. after the last dose, for evaluating passive avoidance task (step-down

latency) and elevated plus maze (transfer latency). The animals were sacrificed for Acetylcholinesterase enzyme estimation. Subgroup 2, 3, receives only treatment dose on induction, whereas subgroup 1 receive only saline no drug no induction. (Table 01)

ExperimentalDesigns



Apparatus

Screening test for memory

Model 1: Passive Avoidance Task (step-down latency) and its disruption by MES and scopolamine were used as two induction methods – used as investigating paradigm

Pole climbing apparatus chamber is used for passive avoidance response where pole is replaced by a wooden platform fixed on electrified grid floor. When rats stepped off the platform, they receive a continuous foot shock from grid floor. The normal reaction of rat was to jump back to the wooden platform. After about 4-5 trials the animals acquired the passive avoidance response and they refrained from stepping down. The criterion was reached when the animal remained on the platform for at least 60s.

Model 2: Elevated Plus maze (Transfer latency) and its disruption by MES and scopolamine were used as two induction methods – used as investigating paradigm

An elevated plus maze consists of two open arms (50 x 10cm) and two closed arms (50 x 10 x 40cm) with an open roof. The maze was elevated to a height of 50cm. the animals were individually placed at the end of either of the open arms and the time taken for the animal to move from open to closed arm (Transfer latency, TL) was noted on the zero day. The animals were allowed to explore the apparatus for 30s. After 24h of the first exposure; TL was again noted on the day-1 of the study for determining the acquisition. The criterion was reached when the animal moved into the closed arms in very short period keeping the cut-off time of 60s (as maximum time taken for moving from open arms to closed one)[16, 17]

Estimation of Acetyl Cholinesterase Enzyme Activity of Discrete Parts of Brain

Dissection

Exactly 60min. after the electroshock and scopolamine treatment the rats were decapitated by Gillette, and the whole brain were taken out quickly. The cerebral cortex, cerebellum, medulla oblongata and midbrain were dissected out as described by Glowinsky and Iverson 1966 suspended in phosphate buffer and weighed accurately.

Preparation of Enzyme Homogenate

Procedure

The different regions of the brain viz. cortex, cerebellum, medulla oblongata and midbrain were homogenized in a tissue homogenizer. [Approximately 20mg of tissue per ml of phosphate buffer pH 7.2].

A 0.4ml aliquot of this homogenate was added to a cuvette containing 2.6ml phosphate buffer (pH 7.2, 0.05M). To this, 100µl of Ellman's reagent and then was taken into the photocell. The absorbance was set to zero at 412nm when the fluctuations stopped.

Of the substrate (Acetyl thiocholine iodide) 20µl was added. A change in the absorbance per minute was noted. The rate of moles of substrate hydrolyzed per minute per gram of tissue was later calculated as per the following equation:

$$R = \frac{\Delta A}{1.36\,(10^4)} \times \frac{1}{(400/3120)\,C_0} = 5.74(10^{-4})\,\frac{\Delta A}{C_0}$$

Where,

ΔA = Change in absorbance per minute (mean change in absorbance from the 1st to 7th min. was taken)

C_0 = Original concentration of the tissue.

R = Rate in moles substrate hydrolyzed per minute per gram of tissue.[18]

Statistical Analysis

The step-down latency and transfer latency were analyzed using the Student's paired't' test, two tailed. Later the inflexion ration was calculated for the transfer latency which was analyzed using One Way Analysis of Variance (ANOVA), followed by Dunnet's't' test for

individual comparison of groups with MES induced, Scopolamine induced and control groups.

The rat brain acetyl cholinesterase activity of different groups were analyzed using One Way Analysis of Variance (ANOVA), followed by Dunnet's 't' test for individual comparison of groups with MES induced, Scopolamine induced and control groups.

RESULTS

In the present study, fruits of *Vitis vinifera* Linn were subjected to phytochemical investigation and cognitive enhancing activity.

Phytochemical investigation

The alcoholic extract when tested for the preliminary phytochemical investigation showed presence of carbohydrates, proteins, cardiac glycosides, alkaloids and flavonoids. After the phytochemical investigation, the alcoholic extract was subjected to TLC. Isolation of compound was done of extracts irrespective of any reason. The isolated compounds were then subjected to characterization and spectral studies such as HPTLC, UV and IR spectroscopy. *Vitis vinifera* showed the similar functional groups as in flavonoids.

Cognitive Enhancement Activity

The alcoholic extracts of the drug was studied for acute oral toxicity study as per OECD/OCDE guidelines. As per the results, alcoholic extracts fruits of *Vitis vinifera* failed to show any signs of toxicity up to 2000 mg/kg body weight. The $1/10^{th}$ of LD_{50} cutoff values were taken for cognitive enhancing activity i.e. 200mg/kg, b.w. alcoholic extracts for both the drugs.

Cognitive Enhancement Activity was done by step down and transfer latency method, impairment of memory consolidation was done by MES & scopolamine. Chronic exposure to MES for 7 days produced a significant decrease in step down latency and increased the time of latency in elevated plus maze .The same effect was seen in scopolamine exposed animals. But both the alcoholic extracts (200mg/kg.B.W) and standard mentats drug showed significant ($P< 0.001$) effect when compare to control, there was significant increase in step down latency and decrease in the of transfers latency which was as effect as that of standard drug (Table 2, Fig-01, 02). This suggested that application of MES and scopolamine disrupts the acquisition, retention and consolidation of learning task which was reversed by alcoholic extracts and standard drug.

In our present study we found that there was significant increase in Acetylcholinesterase enzyme activity in MES exposed and also in scopolamine exposed rats. Whereas; administration of alcoholic extracts of *Vitis vinifera* and mentats simultaneously with MES and scopolamine exposure for 7 days prevented the impairment of memory consolidation and also reduced the Acetylcholinesterase enzyme activity in all parts of brain.(Table 03, Fig- 03)

From the results we have found that the alcoholic extracts of possess *Vitis vinifera* antiamnesic as it reversed the memory impairment produced by MES and Scopolamine

DISCUSSION

As per the results of the spectral studies we can say that the probable structures of the isolated compounds from *Vitis vinifera* Linn. fruit may be flavonoids

The result obtains also showed significant cognitive enhancement activity of alcoholic extract drug. Daily administration of extracts significantly attenuated the amnesic effect of both MES and Scopolamine. The probable mechanism of action could be by cholinergic pathway as it showed a considerable decrease in the level of Acetylcholinesterase enzyme activity.

In conclusion, the study shown that the fruits of *Vitis vinifera* Linn contain flavonoids and hence possess significant cognitive enhancing activity in laboratory animal at dose of 200 mg/kg body weight

However these claims demands further studies such as mass spectroscopy, P-MR and C^{13}-NMR studies to confirm the structure of the compound. Further studies can also be conducted in the transgenic animals and related models of Alzheimer's disease to evaluate the effects of these extracts on Amyloid plaques to establish the other possible mechanism of action in the neuroprotection and memory consolidation

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REFERENCES

1. Vaidyaratnam PS. Varier's Arya Vaidya Sala, Indian Medicinal Plants, A Compendium of 500 Species. Chennai: Orient Longman; 1994;Vol 4: 62-6.
2. Kirtikar KR, Basu BD. Indian Medicinal Plants. 2nd ed. Allahabad: International Book Distributors, Book Sellers and Publishers; 1999; 1: 634,616.
3. Sreemantula S, Nammi S, Kolanukonda R, Koppula S, Boini KM. Adaptogenic and nootropic activities of aqueous extract of *Vitis vinifera* (grape seed): an experimental study in rat model**.** BMC Complementary and Alternative Medicine 2005;5(1): 6882-5.
4. Kotamballi N, Murthy C, Singh RP, Guddadarangavvanahally K, Jayaprakasha J. Antioxidant activities of grape (*Vitis vinifera*) Pomace extracts. Agric Food Chem 2002; 50(21): 5909 -5914.
5. Orhan N, Aslan M, Orhan DD, Ergun E, Yeşilada E. In-vivo assessment of antidiabetic and antioxidant activities of grapevine leaves (*Vitis vinifera*) in diabetic rats . Journal of Ethnopharmacology 2006; 108(2): 280-286.
6. Parekh J, Chanda S. In-vitro antimicrobial activities of extracts of Launaea procumbens Roxb. (Labiateae), Vitisvinifera

L.(Vitaceae) and Cyperus rotundus L.(Cyperaceae). African Journal of Biomedical Research 2006; 9: 89-93.
7. Gharib Naseri MK, Heidari A. Bronchodilatory activity of *Vitis vinifera* leaf hydroalcoholic extract in rat. Iranian Biomedical Journal 2006; 10(2): 79-83.
8. Reddy DS. Assessment of nootropic and amnestic activity of centrally acting agents. Ind J Pharmacol 1997; 29: 208-221.
9. Khandelwal KR, Kokate CK, Purohit AP, Ghokale SB. Practical Pharmacognosy Techniques and Experiments. 9th ed. Pune: Nirali Prakashan; 2002: p. 149-56.
10. Mukherjee PK. Quality Control of Herbal Drugs- An Approach to Evaluation of Botanicals. 1st ed. New Delhi: Business Horizons Pharmaceutical Publications; 2002: p.246-370.
11. Wagner H, Bladt S, Zgainski EM. Plant Drug Analysis- A Thin Layer Chromatography Atlas; Berlin Heiderberg, New York: Springer- Verlag; 1984.
12. Harborne JB. Phytochemical methods- A guide to modern techniques of plant analysis. New York: Champman and Hall, 1984.
13. Stahl E. Thin Layer Chromatography – A laboratory handbook. 2nd ed. Berlin: Heiderberg, New york: Springer-Verlag, 1969.
14. Kemp W. Organic Spectroscopy. 3rd ed. London: Mc millan Press Ltd; 1991.
15. Organization for Economic Co-operation and Development, revised draft Guidelines 423. OECD guideline for the testing of Chemicals. Revised Document. October 2000.
16. Sharma AC, Kulkarni SK. Reversal of scopolamine and dizocilpine induced memory dysfunction by angiotensin converting enzyme inhibitors in rats and mice. Ind J Pharmacol 1992; 24: 147-53.
17. Dhingra D, Parle M, Kulkarni SK. Memory enhancing activity of Glycyrrhiza glabra in mice. J Ethnopharmacol 2004; 91: 361-65.
18. Glowinski J, Iverson LL. Regional studies of catecholamines in the rat brain. I. The disposition of 3H-norepinephrine, 3H-dopamine and 3H-dopa in various regions of the brain. J. Nenrochem. 1966;13, 655-699

Table 01: Grouping of Animals

Main Groups	Sub-groups	
Group I	Normal control	1. No induction and no treatment
Group II	Positive control	*2.* Only *Vitis vinifera* 3. Only Standard
Group III	Negetive control	4. Induction with MES 5. Induction with scopolamine
Group IV	Treatment group Induction with MES + Treatment with extracts	*6. Vitis vinifera* 7. Standard
	Induction with Scopolamine + Treatment	*8. Vitis vinifera* 9. Standard

Table 02. Mean Values of Transfer latency and Step-Down latency of various drug treated groups

Group	S.NO. of Subgroup	Sub Group	Transfer Latency (in Sec.) in Elevated Plus Maze		Step Down Latency (in Sec.) in Step Down Apparatus	
			Before Day 1	After Day 7	Before Day 1	After Day 7
Normal Control	01	Normal control	22.36±1.634	6.492±0.5852	17.41±3.014	25.57±2.700
Positive Control	02	Extract of *Vitis vinifera* Treated	30.44±1.530	17.01±1.048	15.23±1.375	46.28±2.331
	03	Standard (Mentat) Treated	28.89±6.007	19.04±4.447	16.76±1.799	60.00±0.0
Negative Control	04	MES Induced	6.074±1.094	22.18±1.277	19.19±1.443	10.31±0.8922
	05	Scopolamine Induced	24.71±4.325	47.12±5.485	27.43 ± 3.445	11.73±2.402
Treatment Group MES + Drug Treated	06	Extract of *Vitis vinifera* Treated	35.95±1.974	21.61±1.716	15.14±1.496	39.96±1.520
	07	Standard (Mentat) Treated	26.96±3.601	13.03±1.872	15.24±2.522	9.668±1.300
Treatment Group Scopolamine + Drug Treated	08	Extract of *Vitis vinifera* Treated	36.56±1.555	25.16±1.937	20.91±1.740	40.73±3.331
	09	Standard (Mentat) Treated	21.30±9.572	15.48±7.693	32.04±2.235	60.00±0.0

Paired 't' Test Values, Before Vs. After, Two tailed test

Table 03. Mean Values of estimation of Acetylcholinesterase Enzyme activity

Group	S.NO. of Subgroup	Sub-Group	Mean Values of Acetylcholinesterase Enzyme activity (in moles x 10^{-6}/minute/gram of tissue)			
			Cortex	Medulla	Midbrain	Cerebellum
Normal Control	01	Normal Control	7.387 ±0.3078	9.223 ±0.2955	9.783 ±0.5349	3.930 ±0.4251
Positive Control	02	E Vv Treated	4.320** ±0.2718	4.020** ±0.08505	4.517** ±0.3139	1.813** ±0.3583
	03	Standard (Mentat)	3.110** ±0.08327	3.200** ±0.1400	3.437** ±1.032	2.400** ±0.1531
Negative Control	04	MES Induced	9.307 ±0.2143	11.31 ±0.2857	12.53 ±0.1904	7.420 ±0.3205
	05	Scopolamine Induced	8.927 ±0.2392	11.20 ±0.1444	13.58 ±0.1910	6.367 ±0.2834
Treatment Group MES + Drug Treated	06	E Vv Treated	5.140# ±0.3786	4.867# ±0.3495	4.030# ±0.4378	3.440# ±0.2491
	07	Standard (Mentat)	4.887# ±1.178	4.213# ±0.6444	4.130# ±0.6245	1.680# ±0.2117
Treatment Group Scopolamine + Drug Treated	08	E Vv Treated	4.833T ±0.1646	4.950T ±0.3639	5.323T ±0.2512	2.163T ±0.3244
	09	Standard (Mentat)	4.717T ±0.3060	4.743T ±0.4879	4.537T ±0.3606	2.530T ±0.06557

** P<0.01 – Compared to Normal Control
†† P<0.01 – Compared to Normal Control
# P<0.01 – Compared to Negative Control MES Induced
T P<0.01 – Compared to Negative Control Scopolamine Induced.

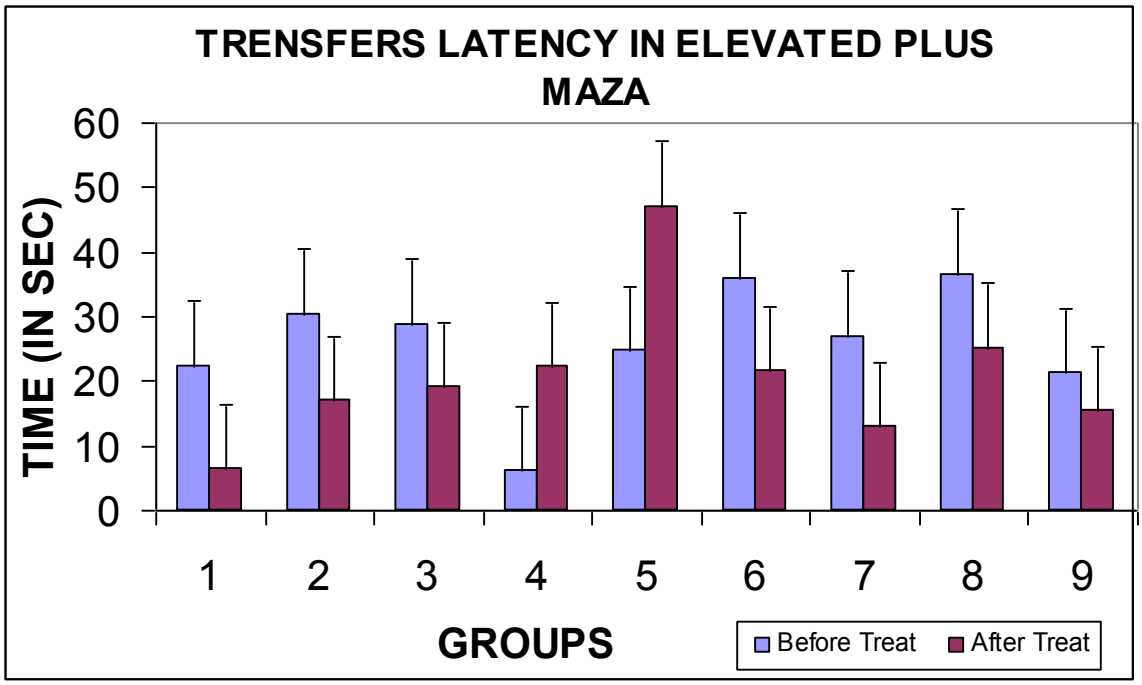

Figure 01: Mean Graph of Transfer Latency

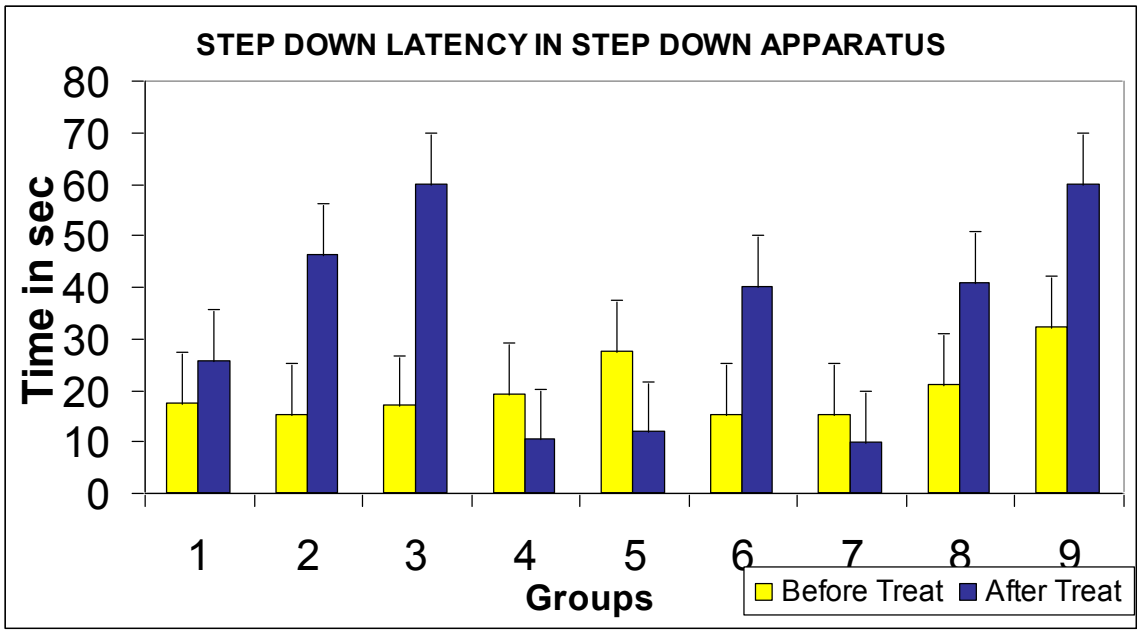

Figure 02: Graph Showing Step Down Latency

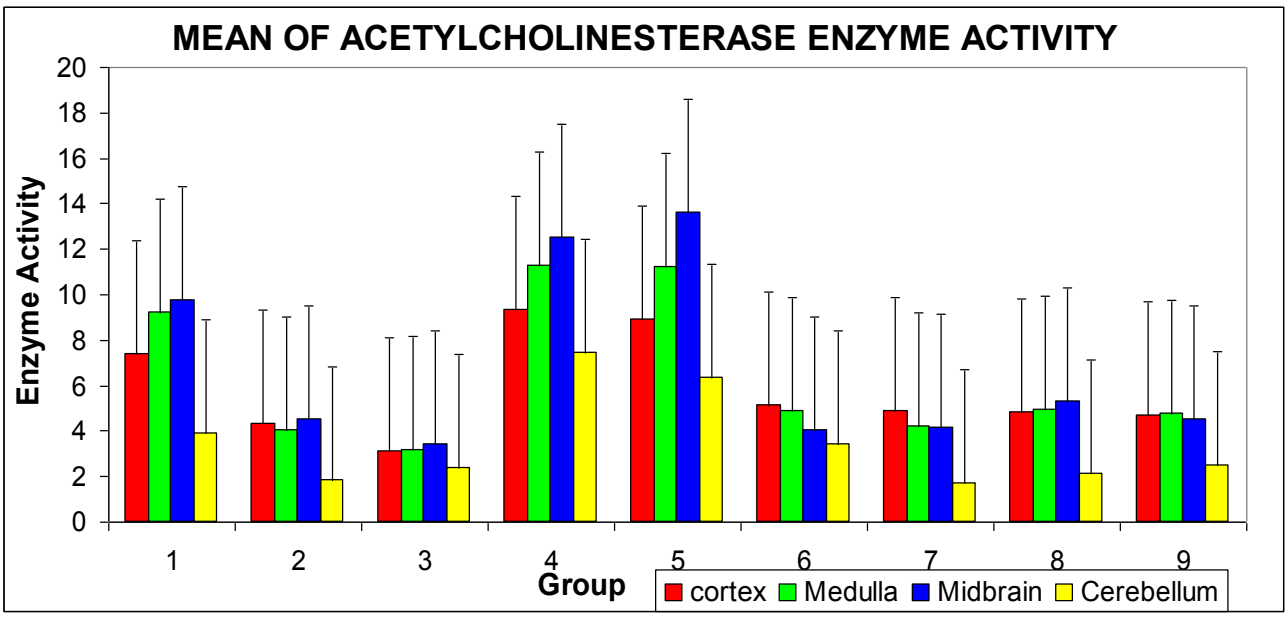

Figure 03: Graph showing Acetylcholinesterase Enzyme Activity

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