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EVALUATION OF COGNITIVE ENHANCING ACTIVITY OF *MIMUSOPS ELENGI* LINN ON ALBINO RATS

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Received: 18-10-2010; Revised: 23-11-2010; Accepted: 30-11-2010

ABSTRACT

In the present study, flowers of *Mimusops elengi* Linn (Sapotacae) commonly known as Bakul was selected for preliminary phytochemical investigation and pharmacological screening of cognitive enhancing activity. The shade dried flowers were subjected to successive solvent extraction with 70 % ethanol. The extracts revealed the presence of carbohydrates, steroids, cardiac glycosides, terpenoids, flavonoids and alkaloids when subjected to chemical tests. TLC was run for their confirmation of terpenoids in *Mimusops elengi* Linn and then 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 maza & 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 of transfers latency and also decrease in acetylcholinesterase enzyme activity but was not as effective as that of standard drug. Hence it can by conclude that the flowers of Mimusops elengi Linn contain triterpenoid and hence possess significant cognitive enhancing activity.

KEYWORDS: *Mimusops elengi* Linn, Phytochemical, cognitive enhancing activity, MES, scopolamine, acetylcholinesterase.

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INTRODUCTION

The plant *Mimusops elengi* Linn (Sapotacae) commonly known as Bakul, Madhugandha, Chirapushpa, Pagademara is highly reputed in traditional medicine as stomachic, astringent, ulemorrhagia. The main chemical constituent present in flower is quarcetin, ursolic acid, D-Mannitol, volatile oil, β-sitisterol. It also contains Quarcetol, ursolic acid, quarcetin and hydroquarcetin and β-sitisterol glycosides present in fruit and seeds. Pulp of fruit contains a large proportion of sugar and saponin. Flowers are also used for preparing lotion for wounds and ulcers. Powder of dried flowers is a brain tonic, expectorant, disease of nose and their smoke is good in asthma. Fruits and seeds are sweet and sour, aphrodisiac, diuretic and good in gonorrhea[1, 2]. It also have found to have hypotensive effect[3], gastric ulcers.[4]

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.[5]

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

MATERIALS AND METHOD

Plant Material

In the present study, flowers of *Mimusops elengi* were collected from local areas of Belgaum, Karnataka. The roots were authenticated from botanist Dr. R. S. Goudar, Department of Botany, R. L. S. Institute, Belgaum, Karnataka, India.

After authentification, the flowers was 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 flowers of *Mimusops elengi* was reduced to fine powder (# 40 size mesh) to obtain a powder of desired particle size. The powder material was subjected to (70%) ethanolic extraction with soxhlet apparatus. 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. [6,7]

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 *Mimusops elengi* extract was Pet. Ether: Acetone (7:3) [8,9,10]

Isolation of Active Principles

The alcoholic extract of *Mimusops elengi* Linn was 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.

Characterization of Isolated compounds

Soon after isolation of compound from *Mimusops elengi* was subjected to detailed characterization for the confirmation of probable structure of the compounds from spectral analysis

U.V. Spectra

The compound *Mimusops elengi* Linn 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 *Mimusops elengi* Linn. and *Vitis vinifera* Linn. were performed using CAMAG TLC scanner 3 and LINOMAT-V. The detailed profiles of HPTLC are given in annexure 1. The isolated phytoconstituents was characterized by spectral studies. [11]

Animals

The Wistar Albino strain rats of either sex weighing 150-250g 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 flowers of *Mimusops elengi* was used as the test drugs for the evaluation of memory enhancing activity.

Acute toxicity study

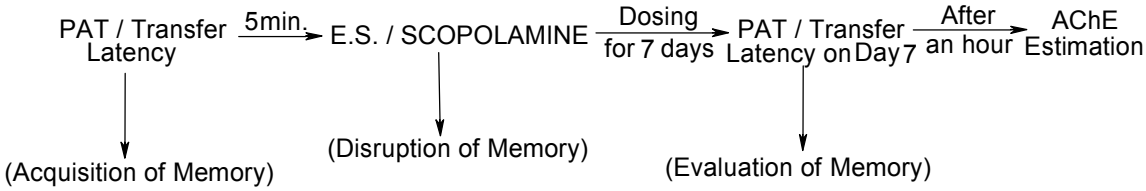
The acute toxicity study was carried out as per the guidelines set by Organisation for Economic Co-operation and Development (OECD) revised draft guidelines 423 B (" Up and Down" method) received from Committee for the Purpose of Control and supervision of Experiments on Animals (CPCSEA), Ministry of 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.[12]

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 7th 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 no. I)

Experimental Designs



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) [13,14]

Estimation of Acetylcholinesterase 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: [15]

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

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.

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, the flowers of *Mimusops elengi* Linn. were subjected to phytochemical investigation and cognitive enhancing activity.

The ethanolic extract when tested for preliminary phytochemical investigation showed presences of carbohydrates, steroids, cardiac glycosides, terpenoids, flavonoids and alkaloids 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. *Mimusops elengi* Linn. showed the similar functional groups as in triterpenes.

The alcoholic extract of the drug were studied for acute oral toxicity study as per OECD/OCDE guidelines. As per the results, alcoholic extracts of flowers of *Mimusops elengi* Linn failed to show any signs of toxicity upto 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 whereas, 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 of transfers latency but was not so effective as that of standard drug (Table no I, 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 m*imusops elengi* Linn. and standard mentat drug 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 no III, Fig- 03)

From the results we have found that the alcoholic extracts of *Mimusops elengi* Linn possess anti amnesic 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 *Mimusops elengi* Linn. flower may be terpenoids.

The result obtain 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 flowers of Mimusops elengi Linn contain triterpenoid 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-NMR 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.

ACKNOWLEDGEMENT

The authors are grateful to Dr. F V. Manvi, Principal KLE's College of Pharmacy, Belgaum and Dr. V G. Joshi, Principal MM's College of Pharmacy, Belgaum for the support this research work

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Table 1: Grouping of Animals

Main Groups	Sub-groups	
Group I	Normal control	1. No induction and no treatment
Group II	Positive control	2. Only *Mimusops elengi*, 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. *Mimusops elengi*, 7. Standard
	Induction with Scopolamine + Treatment	8. *Mimusops elengi*, 9. Standard

Table 2: 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 *Mimusops elengi* Treated	52.82±2.263	32.45±1.140	14.89±1.442	27.82±0.6974
	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 *Mimusops elengi* Treated	19.04±3.749	4.394±0.6521	20.56±0.7858	35.28±2.533
	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 *Mimusops elengi* Treated	37.43±1.665	14.90±1.169	17.46±3.162	41.19±1.782
	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 3: 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 Me Treated	5.017** ±0.1725	4.663** ±0.7255	5.163** ±0.6156	1.983** ±0.1224
	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 Me Treated	5.393# ±0.6376	5.983# ±0.2817	6.160# ±0.4574	3.690# ±0.2350
	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 Me Treated	4.697† ±0.2591	5.830† ±0.4225	6.123† ±0.1650	2.770† ±0.3828
	09	Standard (Mentat)	4.717† ±0.3060	4.743† ±0.4879	4.537† ±0.3606	2.530† ±0.06557

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

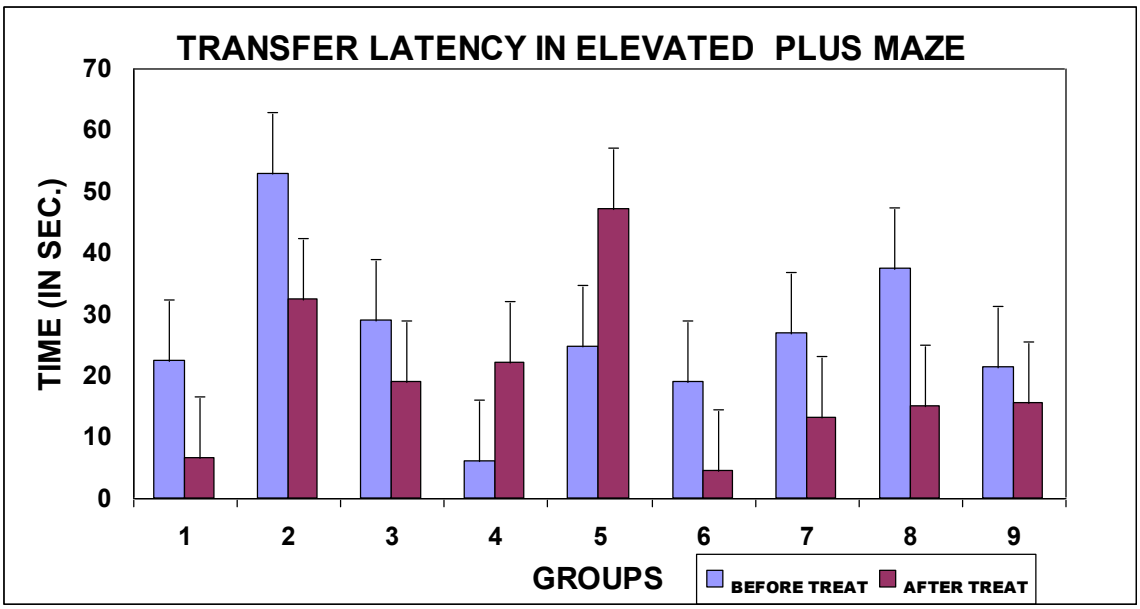

Fig 1: Mean Graph of Transfer Latency

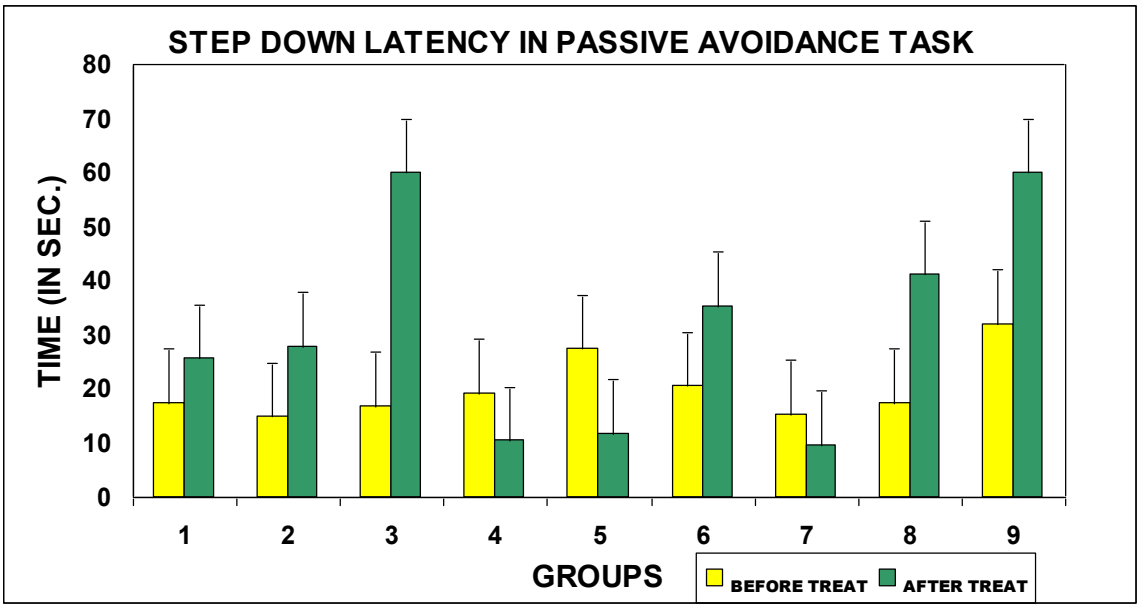

Fig 2: Graph Showing Step down Latency

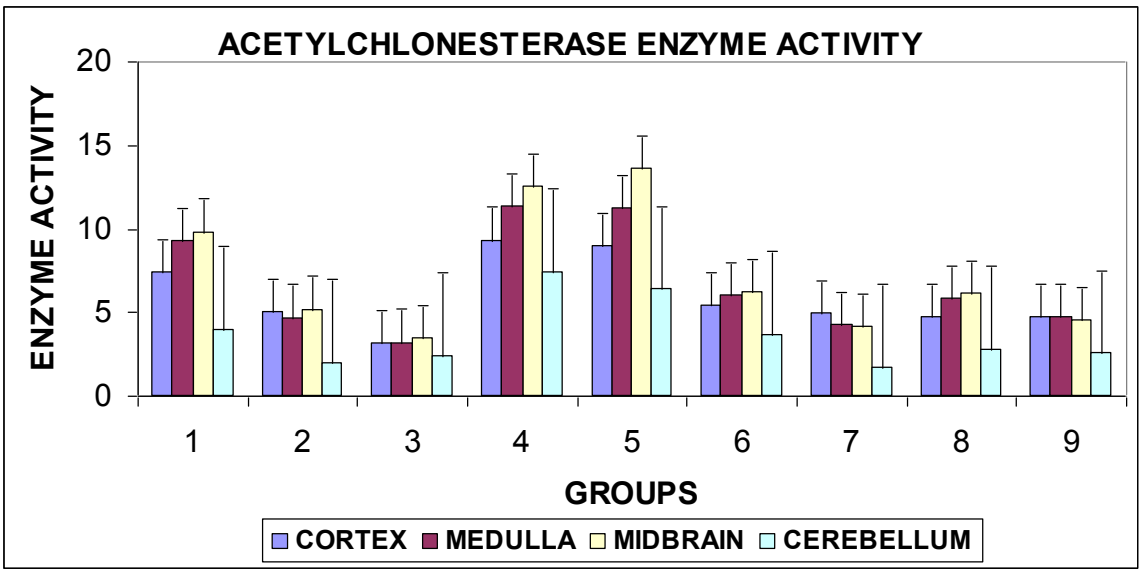

Fig 3: Graph showing Acetylcholinesterase Enzyme Activity

Source of support: Nil, Conflict of interest: None Declared