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NOOTROPIC ACTIVITY OF FRUIT EXTRACTS OF *LITCHI CHINENSIS SONN (SAPINDACEAE)*

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Abstract

In traditional system of medicine the plant is extensively used for different ailments like intestinal and throat problems, general body pain, neuralgic disorders and tonic to brain, heart and liver. Further no scientific data is available on the nootropic activity of fruit extracts of this plant. Hence the present study was aimed to explore the nootropic activity of this plant in experimental animal like mice. Nootropic activity of alcoholic (AEFLC) and aqueous (AQEFLC) extracts of fruit of *L.chinensis sonn* was evaluated in Passive avoidance model (PAM) in mice, Diazepam induced amnesia model (DAM) in mice and Sodium nitrite induced hypoxia model (SHM) in mice. Piracetam was used as standard reference for the all above mentioned models. When these extracts are subjected for LD_{50} studies none of them produced abnormal behaviour or mortality in mice even at the dose level of 2000 mg/Kg body weight. Three different doses like low (100 mg/Kg), medium (200 mg/Kg) and high (400 mg/Kg) doses selected for the present study. For evaluation of nootropic activity, the ALEFLC and AQEFLC were tested at different dose levels and all doses (except low dose of AQEFLC) have shown a significant nootropic effect noted by increased Step-down latency (SDL), decreased time spent in shock zone (TSZ) & number of errors (SDE) in PAM. A significant increase in inflexion ratio was noted with all doses of AEFLC and AQEFLC in DAM. In SHM, AEFLC and AQEFLC have shown a significant increase in time for cessation of respiration.

Keywords: *L.chinensis sonn* fruit, EPM, Shock box, nootropic activity, hypoxia.

Introduction

Nootropics, popularly referred to as “smart drugs” are substances, which boost human cognitive abilities. Typically these are alleged to work by increasing the brain’s supply of neurochemicals, improving brain’s oxygen supply or by stimulating nerve growth[1].

Nootropics represent a new class of psychotropic agents with selective facilitatory effect on integrative functions of the central nervous system, particularly on intellectual performance, learning capability and memory. Nootropic agents such as piracetam, aniracetam and choline esterase inhibitors like donepezil are being used for improving memory, mood and behavior, but the resulting side-effects associated with these agents have made their applicability limited. Indian system of medicine emphasizes use of herbs, nutraceuticals of life style changes for controlling age related neurodegenerative disorders[2].

Alzheimer's disease (AD) is characterised by degenerative changes in the brain accompanied by loss of memory, especially for recent events. The learning and memory is closely associated with the functional status of the central cholinergic system. The basal forebrain provides the major source of cholinergic inputs to the neocortex and hippocampus. The main cholinergic pathways in the mammalian forebrain are the projection from the medial septal nucleus and the nucleus of the vertical limb (diagonal band of Broca) to the hippocampus via the fimbria-fornix and the projection from nucleus basalis cellularis to the neocortex[3]. Despite the severity and high prevalence of this disease, Allopathic system of medicine is yet to provide a satisfactory remedy. Therefore, people are now motivated to explore the Indian traditional system to come up with a promising solution to manage this deadly disease (AD).

The present study was focused upon exploring the potential of an Indian medicinal plant, "*L.chinensis sonn*" for its efficacy in reversing the memory deficits and for its improving acquisition and memory retention in experimental animals.

Materials and Methods

***Drugs*:** Diazepam (Ranbaxy Laboratories Ltd), Piracetam (Micro Labs Ltd,) and Phenytoin (Sun Pharmaceutical India.Ltd) and Sodium nitrite (Merck India Ltd) were used.

***Equipments*:** Elevated plus maze apparatus [Size: open arms (16x5cm), enclosed arms (16x5x12cm)] (Medicraft electro medicals PVT LTD. U.P, India.) and shock box (Techno, Lucknow, India).

***Animals*:** Albino mice of either sex weighing between18-22 procured from Shri Venkateswara Enterprises, Bengaluru for experimental purpose were used in this study. After procuring, the animals were acclimatized for 7 days and housed in groups of 6 under standard husbandry conditions[4,5] like room temperature 26 ± 2^0C, relative humidity 45-55% and light/ dark cycle of 12:12 h. All the animals were fed with synthetic standard diet (Amrut

Laboratories Pranava, Agro Industries Ltd. Sangli, Maharashtra) and water was supplied *ad libitum* under strict hygienic conditions. After obtaining permission from Institutional Animal Ethical Committee (IAEC) of V. L. College of Pharmacy, Raichur (Karnataka), animal studies were performed as per rules and regulations in accordance to guidelines of CPCSEA with registration number 557/02/c/CPCSEA.

Animals were fasted overnight prior to vehicle/standard/extract administration and during the experiment. All experiments were carried out during the light period (8:00 to 16:00 h).

Preparation of different extracts with fruit of *litchi chinensis* sonn

Alcoholic extract: The fruit collected was dried under shade was powdered to coarse powder. The dried powder (200 g) was taken into 1L round bottom flask and 95% alcohol in sufficient quantity was added, then it was kept for maceration for 72 h. The extract was filtered to obtain AEFLC and concentrated on a water bath maintained at 45^0 C to get a solid mass and extract was stored in an airtight container in a refrigerator below $10^{\circ}C$[6].

Aqueous extract: The aqueous extract of fruit powder was prepared by macerating coarse powder with distilled water (containing 2% chloroform (10 ml), which acts as preservative) for 24 h, and was filtered and concentrated on a water bath maintained at 45^0 C to get a solid mass of AQEFLC and extract was stored in an airtight container in a refrigerator below $10^{\circ}C$[6].

Preliminary phytochemical investigation

Preliminary phytochemical investigation of both AEFLC and AQEFLC were noted with carbohydrates, flavonoids, tannins, triterpenes, Vitamin C, proteins and amino acids[7, 8,9,10].

Determination of LD_{50} of AEFLC and AQEFLC

The acute toxicity of both the extracts was determined by using albino mice of either sex (18-22 g) maintained under standard husbandry conditions were fasted for 3 h prior to the experiment. Animals were administered with single doses of either AEFLC or AQEFLC and observed for their mortality up to 48 h study period (short term toxicity). Based on the short-term toxicity profile, the next dose of each extract was determined as per OECD Guidelines No 425. From the LD_{50} dose of individual extracts 1/20, 1/10 and 1/5 th doses selected and considered as low, medium and high dose respectively and were used in the entire study[11].

Experimental protocol

1. Passive avoidance model in mice (Exteroceptive behaviour model)

Passive- avoidance task is a method widely used for screening drugs affecting learning and memory. The method described by Papazova et al, 1994 was modified as follows. An inverted petridish placed in the centre of the grid floor of a continuous avoidance apparatus (Techno, Lucknow) was used. The petridish served as the shock-free zone (SFZ). Mice were placed in the SFZ and up on stepping down from the SFZ were given an electric shock (20 V) through the grid floor. Animals were trained to remain on the SFZ for at least 60 sec and mice which did not meet these criteria in 5 trials were rejected. Observations were made for acquisition i.e. the number of trials required to reach the learning criteria and for retention of learning for 10 min at 2 h and 24 h post-training. The following retention parameters like step-down latency (SDL) in seconds, step-down error (SDE) as the number of times the animal stepped down from the SFZ and the time spent in the shock zone (TSZ) in seconds are noted[12].

Treatment schedule: The memory- impairing dose of phenytoin 25 mg/kg was administered daily once for 14 days and the selected doses of AEFLC and AQEFC for 7 days i.e. on 8^{th} to 14^{th} day and the parameters mentioned above were noted. Group I was maintained as normal control which was given with distilled water (10 ml/kg, p.o.), Groups II, III, IV, V, VI, VI.VIII and IX with phenytoin (25 mg/kg p.o.) daily once for 14 days. Additionally group III with piracetam (200 mg/kg, p.o.) 8^{th}-14^{th} which served as standard, Groups IV, V,VI and Groups VII,VIII,IX were treated with different doses of AEFLC (100, 200 and 400 mg/kg p.o.) and AQEFLC (100, 200 and 400 mg/kg p.o.) respectively daily once for 7 days as mentioned above.

2. Diazepam induced amnesia in mice (Interoceptive Behaviour models)

All groups were treated respectively as mentioned below for a period of 15 days and Diazepam 1 mg/kg was given i.p 90 min after the last dose of standard/ AEFLC and AQEFLC to induce impairment of memory that act through GABAergic system. Transfer latency (TL) was recorded with Elevated Plus maze (EPM) at 2 h and 24 h after injection of Diazepam. The inflexion ratio was calculated by the following formula: $IR = (L_0 – L_t) / L0$

Where L_0 – intial transfer latency on the 15^{th} day.

L_t – Transfer latency on 16^{th} day[13,14].

Treatment schedule

Group I was maintained as normal control which was given with distilled water (10 ml/kg, p.o.), Group II with Diazepam alone (1mg/kg, i.p) only on 15^{th} day, Group III with piracetam (200 mg/kg, p.o.) which served as standard and Groups IV, V, VI and VII, VIII, IX were treated with different doses of AEFLC (100,200 and 400

mg/kg p.o.) and AQEFLC (100,200 and 400 mg/kg p.o.) respectively and after 90 min of the last dose for all the groups from III, IV, V, VI, VII, VIII, IX were given with Diazepam (1 mg/kg, i.p).

3. Sodium nitrite intoxication in mice (Metabolic Influence model)

All the groups were treated according to the protocol as mentioned below for a period of 7 days and sodium nitrite 250 mg/kg was given s.c 60 min after last dose of standard/ AEFLC and AQEFLC to induce chemical hypoxia. Sodium nitrite reduces the oxygen carrying capacity of the blood by converting hemoglobin to methemoglobin and cessation of respiration time in each group of mice was recorded[15].

Treatment schedule:

Group I was maintained as normal control which was given with distilled water (10 ml/kg, p.o.), Group II with Sodium nitrite alone (250 mg/ kg s.c) daily once for 7 days, Group III with piracetam (200 mg/kg, p.o.) which served as standard, Groups IV, V, VI and VII, VIII, IX were treated with different doses of AEFLC (100,200,400 mg/kg p.o.) and AQFLC (100,200,400 mg/kg p.o.)

Statistical Analysis: The results are expressed as the mean ± SEM, and were analyzed using one-way ANOVA followed by Dunnett's multiple comparison tests. Data was computed for statistical analysis by using Graph Pad INSTAT and PRISM Software.

Table No .1. Effect of AEFLC and AQEFLC on Passive avoidance learning and retention (Shock box model) in mice

Treatment	Dose/kg (p.o)	Step-down latency Trail	Step-Down atency (SDL)		Step-Down Error (SDE)		Time spent in shock zone (TSZ)	
			Learning	Retention	Learning	Retention	Learning	Retention
Normal control (Vehicle)	10 ml	3.0	70.00±5.32	114.16±7.12	3.16±0.30	2.00±0.25	25.83±1.77	17.16±1.40
Toxicant Control (Phenytoin)	25 mg	3.3	20.83±1.53	28.50±3.54	5.16±0.47	4.33±0.33	31.50±1.17	20.00±1.89
Standard (Piracetam)	200 mg	3.0	147.83±9.48**	277.16±10.69**	1.66±0.21**	1.00±0.25**	10.16±0.47**	4.00±0.36**
AEFLC	100 mg	3.16	113.30±7.14*	195.83±7.57**	2.00±0.25*	1.00±0.25**	20.33±1.40*	14.00±1.30**
AEFLC	200 mg	2.66	128.83±10.42**	225.00±13.03**	1.83±0.16**	0.83±0.16**	13.66±0.88**	8.66±1.70**
AEFLC	400 mg	3.1	140.83±8.50**	246.00±11.64**	1.50±0.22**	0.50±0.22**	9.66±0.80**	5.66±0.71**
AQEFLC	100 mg	2.83	117.00±4.47*	180.00±7.85**	4.16±0.30^{ns}	3.00±0.36^{ns}	28.50±1.088^{ns}	20.83±0.94 ^{ns}
AQEFLC	200 mg	3.3	130.33±7.12**	204.16±13.68**	1.83±0.30**	1.00±0.25**	18.83±1.13**	13.33±1.22**
AQEFLC	400 mg	3.16	132.50±12.63**	231.66±18.64**	1.50±0.22**	0.83±0.16**	12.33±0.66**	6.33±0.55**

n = 6, Significant at P < 0.05*, 0.01** and 0.001***, ns = not significant.

AEFLC-Alcoholic extract of fruit of *L.chinensis sonn.* AQEFLC-Aqueous extract of fruit of *L.chinensis sonn.*

Fig No .1. Effect of AEFLC and AQEFLC on SDL (Shock box model) in mice.

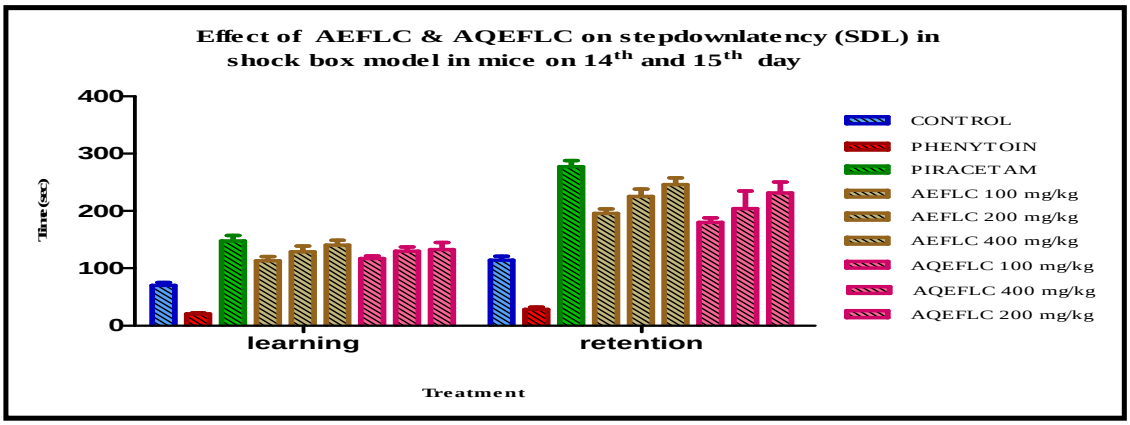
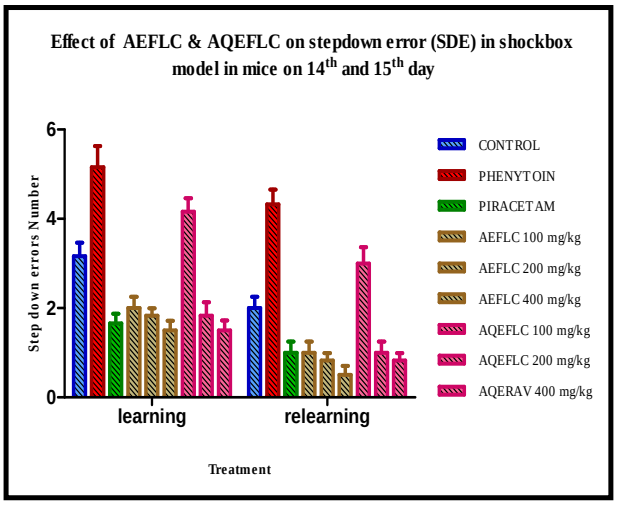


Fig No .2. Effect of AEFLC and AQEFLC on SDE and TSZ (Shock box model) in mice.



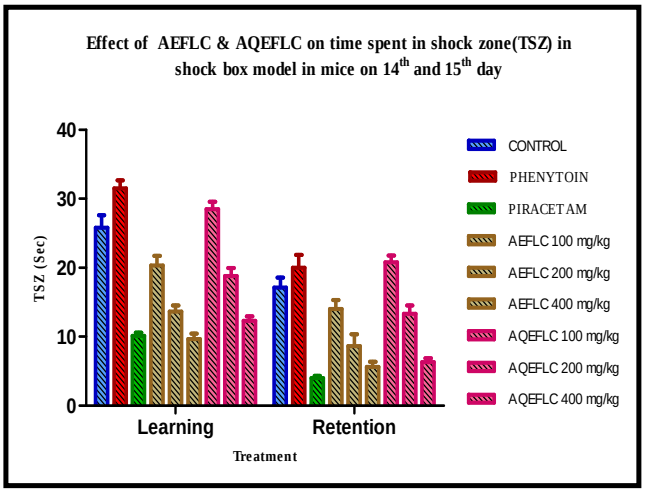


Table No .2. Effect of AEFLC and AQEFLC on inflexion ratio in Diazepam induced Amnesic Model in mice.

Treatment	Dose/ kg	Inflexion Ratio (mean±SEM)
Normal control (vehicle)	10 ml p.o.	0.379±0.016
Diazepam alone	1.0 mg i.p.	0.270±0.015
Piracetam + Diazepam	200 mg p.o.+ 1.0 mg i.p	0.670±0.016**
AEFLC + Diazepam	100 mg p.o. + 1.0 mg i.p.	0.459±0.010**
AEFLC + Diazepam	200 mg p.o. + 1.0 mg i.p.	0.567±0.014**
AEFLC + Diazepam	400 mg p.o. + 1.0 mg i.p.	0.634±0.018**
AQEFLC + Diazepam	100 mg p.o. + 1.0 mg i.p.	0.443±0.018*
AQEFLC + Diazepam	200 mg p.o. + 1.0 mg i.p.	0.553±0.015**
AQEFLC + Diazepam	400 mg p.o. + 1.0 mg i.p.	0.604±0.016**

n = 6, Significant at P < 0.05*, 0.01** and 0.001***, ns = not significant.

AEFLC-Alcoholic extract of fruit of *L.chinensis sonn,* AQEFLC-Aqueous extract of fruit of *L.chinensis sonn.*

Fig No .3. Effect of AEFLC and AQEFLC on inflexion ratio in Diazepam induced Amnesic Model in mice.

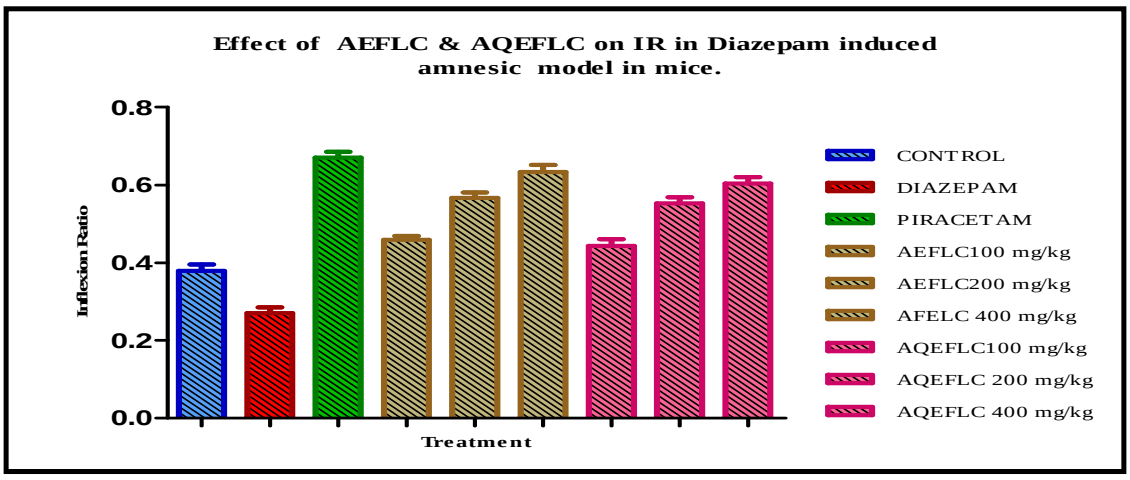
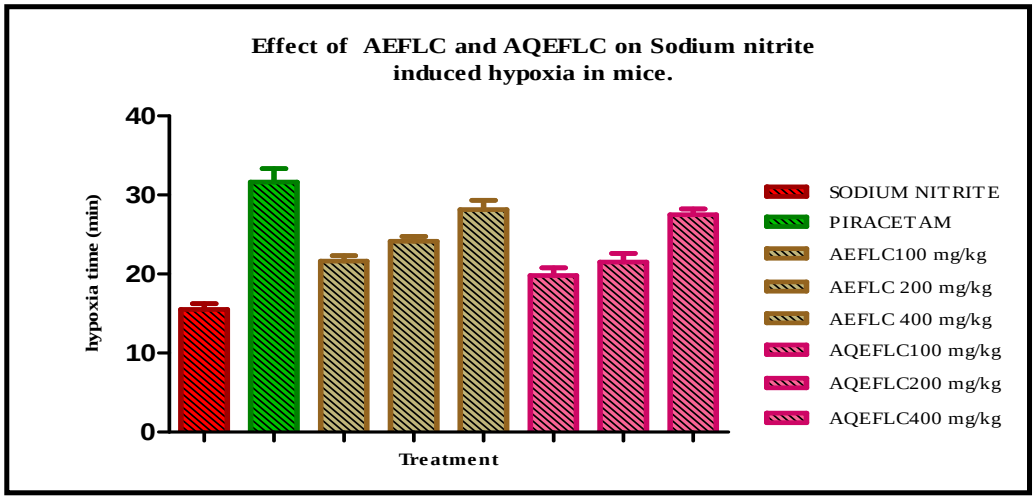


Table No .3. Effect of AEFLC and AQEFLC on Sodium nitrite induced hypoxia model in mice.

Treatment	Dose/kg	Time taken for the cessation of respiration
Sodium nitrite	250 mg i.p.	15.50±0.76
Piracetam	200 mg p.o	31.66±1.70**
AEFLC	100 mg p.o	21.66±0.66**
AEFLC	200 mg p.o	24.16±0.60**
AEFLC	400 mg p.o	28.16±1.19**
AQEFLC	100 mg p.o	19.83±0.94*
AQEFLC	200 mg p.o	21.50±1.11**
AQEFLC	400 mg p.o	27.50±0.76**

n = 6, Significant at P < 0.05*, 0.01** and 0.001***, ns = not significan AEFLC-Alcoholic extract of fruit of *L.chinensis sonn.* AQEFLC-Aqueous extract of fruit of *L.chinensis sonn.*

Fig No.3. Effect of AEFLC and AQEFLC on Sodium nitrite induced Hypoxia model in mice Results and discussion.



1. Effect of AEFLC and AQEFLC on passive avoidance learning and retention in mice

The results indicate that Piracetam (200 mg/kg)/AEFLC (100, 200 and 400 mg/kg) / AQEFLC (200 and 400 mg/kg) treatment groups has taken less number of trials and less time to reach the criterion of conditioned avoidance learning. When compared to normal group of animals the number of trials and time to reach the criterion of conditioned avoidance learning were significantly decreased by piracetam/AEFLC/AQEFLC and Piracetam and all three doses of AEFLC, AQEFLC (except 100 mg/kg) have shown a significant nootropic activity.

2. Effect of AEFLC and AQEFLC on inflexion ratio in mice (Diazepam-induced amnesic model)

The elevated plus maze is used to measure the anxiety state in animals, however transfer latency (TL) i.e. the time elapsed between the movement of the animal from an open arm to an closed arm markedly shorten if the animal has previous experience of entering from an open arm to a closed arms and this shortened TL has been shown to be related with memory processes. In EPM acquisition (learning) can be considered as transfer latency on 1st day trials and the retention/ consolidation (memory) is examined 24 h later[16]. In Diazepam induced amnesia model, Diazepam a GABA mimetics agent induces memory impairment and it was reported that a subsequent inhibition of GABA-B receptors has been found to facilitate learning and memory[17]. Diazepam (1mg/Kg) prolonged TL and decreased IR. The AEFLC (100, 200 and 400 mg/Kg) and AQEFLC (100, 200 and 400 mg/Kg) have decreased TL and increased IR thus confirmed their nootropic activity. The protective effect offered by these two extracts against Diazepam-induced amnesic model may be due to facilitating the release of Ach or inhibition of cholinesterase enzyme in the brain.

3. Effect of AEFLC and AQEFLC on Sodium nitrite intoxication model in mice

In Sodium nitrite intoxication model, the Sodium nitrite converts haemoglobin to methemoglobin, thereby reducing oxygen carrying capacity of the blood and cholinergic transmission and ultimately leading to death[18]. Piracetam (200mg/kg)and AEFLC (100, 200 and 400 mg/kg) /AQEFLC (100, 200 and 400 mg/kg) has prolonged th e time taken for cessation of respiration against Sodium nitrite induced hypoxia. The AEFLC/AQEFLC extracts might have reduced the conversion of hemoglobin to methemoglobin and increased oxygen-carrying capacity.

Phytoconstituents like steroidal/triterpenoid saponins, alkaloids, flavonoids, tannins and glycosides from different plants like *Albizzia lebbeck, Panax ginseng, Red ginseng, Bacopa monniera, Asparagus racemousus, Rubia cardifolia, Vinca minor, Secale cornutum, Pueraria tuberose, Vitis vinifera, Guarana and Withania*

somnefera are already reported for their nootropic activity. In the present study it was found that both AEFLC and AQEFLC contained some of these phytoconstituents like flavonoids, tannins, triterpenes, hence these may be responsible for the observed nootropic activity in different experimental animal models used in the study.

Conclusion

Preliminary phytochemical investigation with both the extracts positively responded for the presence of carbohydrates, flavonoids, tannins, triterpenes, Vitamin C, proteins and amino acids. Acute toxicity (LD_{50}) studies with AEFLC and AQEFLC confirmed no abnormal behaviour or mortality at the dose limit of 2000 mg/Kg body weight. Nootropic activity of the AEFLC and AQEFLC were tested at different dose levels and all doses (except low dose of AQEFLC) have shown a significant nootropic effect by increased Step-down latency (SDL), decreased time spent in shock zone and number of errors in Passive avoidance paradigm. A significant increase in inflexion ratio was noted with all doses of AEFLC and AQEFLC in Diazepam induced amnesia model. In Sodium nitrite intoxication model, AEFLC and AQEFLC have shown a significant increase in cessation of respiration time in mice.

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