EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 6
Issue: 7
162-168
Year: 2019

EVALUATION OF NEUROPROTECTIVE PROPERTY OF *RAUWOLFIA SERPENTINA* IN HIGH FAT DIET (HFD) AND STREPTOZOTOCIN (I.C.V) INDUCED MEMORY IMPAIRMENT IN MICE

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Article Received on 26/04/2019 **Article Revised on 16/05/2019** **Article Accepted on 06/06/2019**

ABSTRACT
The present study was designed to evaluate the Neuroprotective property of *Rauwolfia serpentina* in High Fat Diet (HFD) and Streptozotocin (ICV) induced Memory impairment in mice. Eleven groups of mice and each group comprising of six animals were employed in the present study. Different doses of Rauwolfia serpentina (10, 30, 60 mg/kg) was administered during experiment. Mice were treated with i.c.v streptozotocin and kept on High Fat diet for 90 days to induce memory impairment. A methanolic extract (95%) of *Rauwolfia serpentina* enriched with polyphenols was prepared. Memory was evaluated by using Morris Water Maze (MWM) and Elevated plus Maze (EPM). At the end of the study, total serum cholesterol level, Lipid Peroxidation level, Reduced Glutathione level and Brain total protein level were estimated. Administration of *Rauwolfia serpentina* (RS) attenuated HFD and STZ induced rise in ELT and HFD induced decrease in time spent in target quadrant in MWM. Initial transfer latency (ILT) did not differ significantly in any of the groups whereas administration of *Rauwolfia serpentina* (RS) lowered the retention transfer latency (RTL) in HFD and STZ treated mice. Administration of RS produced significant decrease in STZ induced brain oxidative stress (increase in lipid Peroxidation (LPO), decrease in reduced glutathione level). On administration of RS, high fat diet treated mice also produced significant reversal of elevated serum cholesterol levels and memory impairment. These finding suggest that *Rauwolfia serpentina* may have a promising role as prophylactic agent in HFD and streptozotocin induced experimental dementia due to its Neuroprotective property.

KEYWORD: Dementia**,** Streptozotocin, Neuroprotective, Polyphenols.

INTRODUCTION
Alzheimer's disease (AD), is first characterized by Alois Alzheimer in 1907, and is a gradually progressive dementia affecting both cognition and behavior.[1] The loss of cholinergic neurons in the hippocampus and frontal cortex is a feature of disease and thought to underlie the cognitive deficit and loss of memory that occur in AD. The amyloid hypothesis suggests that a fault with the processing of amyloid precursor protein (APP) in the brain leads to the production of a short fragment of APP known as beta-amyloid. The theory rests on the idea that there is the accumulation of this sticky protein fragment in the brain which triggers the disruption and destruction of nerve cells that causes Alzheimer's disease. The discovery of vast cholinergic cell loss lead to development of a cholinergic hypothesis linked to the pathophysiology of Alzheimer disease. The cholinergic hypothesis targeted cholinergic cell loss as the source of memory and cognitive impairment in Alzheimer disease.[1] A growing amount of evidence underscores a mechanistic link between cholesterol and the occurrence of Alzheimer disease. Elevated cholesterol level has been associated with the increased formation and deposition of amyloid beta peptide from amyloid precursor protein, whereas drug that inhibited cholesterol synthesis, lowered A-beta. Oxidative stress is the characterized by an imbalance in radical production of reactive oxygen species (ROS) and antioxidative defense, both are considered to have a major role in the process of age related neurodegeneration and cognitive decline.[2-6]

Plant profile: *Rauwolfia serpentina* L. Benth. Ex Kurz possesses an important role in the pharmaceutical world due to the presence of large number of therapeutic properties. Various secondary metabolites are present in plant which includes alkaloids, carbohydrates, flavonoids, phlobatannins, resins, saponin, tannins and terpenes.[8-10] Other constituents are alcohol, organic acid, polyphenols/phenolic acid, aldehyde, ketone, alkaline, pyrimidine, indole, phytosterol fatty acid and dicarboxylic acid.

MATERIAL AND METHOD

Extraction of active constituents from *R.serpentina* root

Extraction was carried out with methanol (95%) as solvent using soxhlet apparatus for 48 hr and extract was dried under room temperature and stored under 10^{o} c temp. until used.

Eleven groups of mice and each group comprising of six animals. 1: Control. Normal saline (10 ml/kg, i.p) administered by intraperitoneal route 30 min before conducting trials in MWM and EPM test for 25 days. 2: streptozotocin (3 mg/kg, i.c.v). Mice injected intracerebroventricularly Sstreptozotocin in two dosage regimen i.e. on first day and third day followed by exposure to MWM AND EPM test after 15 days.3: streptozotocin (3mg/kg i.c.v) + vitamin E (100 mg/kg) administered in diabetic animal for 15 days. 4: streptozotocin (3mg/kg i.c.v) + *Rauwolfia serpentina* (methanolic extract)(10 mg/kg) administered in diabetic animal for 15 days.5: streptozotocin (3mg/kg i .c.v) + *Rauwolfia serpentina* (methanolic extract) (30 mg/kg) administered in diabetic animal for 15 days.6: streptozotocin (3mg/kg i.c.v) + *Rauwolfia serpentina* (methanolic extract) (60 mg/kg)administered in diabetic animal for 15 days.7: High fat diet Control. Mice were access to the HFD 24 hrs/ day for 90 days and then subjected to MWM and EPM test.8: High fat diet + Atorvastatin (10 mg/kg/d). HFD fed mice administered Atorvastatin for 15 days and then for 5 days during exposure to MWM and EPM test. 9: High fat diet + *Rauwolfia serpentina* (10 mg/kg) administered in animal for 15 days and then 5 days during exposure to MWM and EPM test.10: High fat diet + *Rauwolfia serpentina* (30 mg/kg) administered in animal for 15 days and then 5 days during exposure to MWM and EPM test.11: High fat diet + *Rauwolfia serpentina* (60 mg/kg) administered in animal for 15 days and then 5 days during exposure to MWM and EPM test.

Morris water Maze: The maze consisted of a circular pool, 120 cm in diameter and 50 cm in height, filled to a depth of 30 cm with water at 25 _ 2 8C. Each animal was subjected to four consecutive training trails on each day with inter- trial interval of 5 min. The starting position was changed for each trial while the target quadrant (Q4) remained same. The mice were allowed 120 seconds to locate the submerged platform and upon finding the platform, rats were allowed to stay on the platform for 20 sec. If mice failed to reach the platform within 120 seconds, then they were guided onto the platform and allowed to remain there only for 20 sec. The time taken by the mice to locate the hidden platform in the water maze is known as escape latency time (ELT). Mice were subjected to training trials that is acquisition for four consecutive days. The platform was removed on day fifth and each mouse was allowed to explore the pool for 120 sec. Mean time spent in all the four quadrants was noted down. The mean time spent by the mice in the target quadrant in search of hidden platform was noted as an index of retrieval. The position of experimenter should always remain constant in each trial.

Elevated Plus Maze:[12] The apparatus consist of two open arms which is 16 cm x5 cm and two closed arms of size 16cmx5cmx12cm. On the first day, each mouse was placed at one end of an open arm facing away from the central platform. The time taken by the mouse to move from an open arm to any one of the closed arm with all its four legs in is termed as transfer latency. Transfer latency measured on the plus maze on first day served as the index of learning and acquisition, whereas the transfer latency on second day served as the index of retrieval and memory.

BIOCHEMICAL PARAMETER FOR EVALUATION OF LEARNING AND MEMORY

Collection Of Blood Sample

Blood sample was collected from the retro- orbital plexus of mice under effect of light anesthesia.[15]

Estimation Of Serum Total Cholesterol

The total serum cholesterol level was determined by using Allain method with slight modification by employing commercially available standard cholesterol estimation kit. The blank, standard and test sample was prepared according to the standard procedure as mentioned in the standard cholesterol estimation kit. The absorbance was measured against blank 540 nm using spectrophotometer.[16-18]

Collection of Brain Samples

Animals were sacrificed by cervical dislocation and then the brain were removed and homogenized in phosphate buffer (pH-7.4). The homogenates were then centrifuged at 3000 rpm (i.e., at 2000xg) for 15 min.[20-22]

Estimation of lipid Peroxidation (LPO): It was estimated by using the method described by slater and sawyer (1971).[21] Lipid Peroxidation is assessed indirectly by the measurement of the secondary products, like malondialdehyde (MDA). The most common method of measuring MDA is based on its reaction with TBA. MDA reacts with TBA at high temperature in acidic condition. The reaction yields a pink MDA-TBA adduct. This colored complex can be measured by spectrophotometric ally at 532 nm.

Estimation of reduced Glutathione

Reduced glutathione was estimated by the method of Ellman GL. Glutathione consist of some sulphydryl groups. 5,5 dithio bis 2 nitro benzoic acid (DTNB), a disulphide compound gets easily attacked by tissue sulphydryl groups and form yellow colored anion which is measured at spectrophotometer at 412 nm.

Protein estimation[21-23]

Protein values were estimated to express the biochemical parameters in terms of per mg protein. The quantitative

measurement of the total protein was determined by using Lowry's method.

Statistical Analysis

All results were expressed as mean ± SEM. Data was analyzed by using one way ANOVA followed by Tukey's test and Bonferroni Test. P< 0.01 and p< 0.05 was considered to be statistically significant.

RESULTS

Effect of HFD, STZ and *Rauwolfia serpentina* on Escape latency Time (ELT) and Time Spent in Target Quadrant (TSTQ) during acquisition and retrieval trial using Morris Water Maze

Control group (saline treated) showed significant decrease (p<0.001) in day 4 ELT as compared to its ELT on day 1 (Table 5.1). Further, these mice spent significantly (p<0.001) more time in the target quadrant(Q4) in search of missing platform as compared to the time spent in other quadrants (Q1, Q2, Q3) on water maze(Table 5.1). HFD and STZ control group showed a significant (p<0.001) increase in ELT as compared to ELT of control group (Table 5.1), reflecting impairment of acquisition. Furthermore, HFD and STZ treated mice also showed significant (p<0.001) decrease in time spent in target quadrant (TSTQ) in search of missing platform on water maze (Table 5.2), reflecting impairment in retrieval memory. Administration of *Rauwolfia serpentina* (10, 30, 60 mg/kg, p.o) significantly(p<0.01) attenuated HFD and STZ induced rise in ELT(Table 5.2) and HFD and STZ induced decrease in time spent in target quadrant (p<0.01) (Table 5.2) as compared to HFD control group, indicating reversal of HFD and STZ induced learning and memory deficits. Treatment with Atorvastatin and vitamin E significantly (p<0.01) attenuated the HFD and STZ induced increase in ELT (Table 5.1, 5.2).

Table 5.1: Effect of HFD, STZ and RS on Escape Latency Time (ELT) during Acquisition Trials in Morris Water Maze.

GROUP	DAY 1	DAY 2	DAY 3	DAY 4
Control	98±0.96	69±0.98	56±0.91	48±0.97
HFD Control	108±0.94	79±0.94	69±0.95	57±0.98
HFD + Atorvastatin	81±0.95	69±0.95	58±0.93	52±0.92
HFD + RS (10mg/kg)	102±0.94	69±0.97	57±0.94	54±0.97
HFD + RS (30 mg/kg)	100±0.92	64±1.24	54±0.98	53±0.94
HFD + RS (60 mg/kg)	96±0.94	56±0.93	52±0.92	48±0.96
STZ Control	110±0.91	99±0.93	87±0.97	72±1.21
STZ + Vitamin E	81±0.95	73±0.94	62±0.94	58±0.92
STZ + RS (10mg/kg)	102±0.99	78±0.97	70±1.42	56±0.97
STZ + RS (30 mg/kg)	100±0.93	74±1.21	62±0.91	53±0.93
STZ + RS (60 mg/kg)	99±0.95	69±0.93	54±0.94	49±1.24

Each group (n=6) represent mean ± standard errors of means (S.E.M)
a= p< 0.01 Vs. ELT on same day of control group.
b= p< 0.01 Vs. ELT on same day of control group

Table 5.2: Effect of HFD, STZ and RS on Time spent in Target quadrant (TSTQ) during Retrieval trial in Morris water maze.

Group	Q1	Q2	Q3	Q4
Control	17±0.92	15±0.97	16±0.92	72±1.21[a]
HFD Control	26±1.22	20±1.92	36±1.99	38±2.33[b]
STZ Control	29±1.51	21±1.47	31±1.48	39±1.32[b]
HFD + Atorvastatin	17±0.92	18±0.96	19±0.91	66±0.98[b]
HFD + RS (10mg/kg)	20±0.96	20±1.48	18±1.32	62±1.21[b]
HFD + RS (30 mg/kg)	21±0.92	15±0.97	18±0.92	65±0.98[b]
HFD + RS (60mg/kg)	20±0.99	16±1.33	19±1.52	65±1.69[b]
STZ + Vitamin E	21±0.94	19±0.96	15±0.91	65±0.96[b]
STZ + RS (10mg/kg)	19±0.96	22±1.46	19±1.42	60±1.31[b]
STZ + RS (30 mg/kg)	17±0.91	18±0.98	18±0.94	67±0.92[b]
STZ + RS (60mg/kg)	23±0.97	20±1.23	18±0.96	59±0.94[b]

Each group (n=6) represent mean ± standard errors of means (S.E.M)
a= p< 0.01 Vs. time spent in other quadrant in control group.
b= p< 0.01 Vs. time spent in target quadrant i.e Q4 of control group.

Effect of HFD, STZ and RS on Retention Transfer Latency (RLT) using elevated plus Maze

Initial transfer latency (ILT) did not differ significantly in any of the groups. Retention transfer latency (RTL) of HFD control and STZ control group significantly ($p<0.05$) increased as compared to control group, indicating significantly impairment in learning and memory (Table 5.3). Administration of *Rauwolfia serpentina* (10, 30, 60 mg/kg p.o) significantly ($p<0.05$) lowered the RTL in HFD treated mice, indicating reversal of HFD induced learning and memory deficits. Treatment with Atorvastatin significantly ($p< 0.05$) (Table 5.3) lowered the RTL in HFD treated mice indicating improvement of learning and memory.

Table 5.3: Effect of HFD and STZ on Retention transfer Latency (RTL) using Elevated Plus Maze.

Group	Retention Transfer Latency(in sec)
Control	42±0.91
HFD Control	51±1.22[a]
STZ Control	57±2.32[a]
HFD + Atorvastatin	32±1.28[a]
HFD + RS (10mg/kg)	24±0.99[b]
HFD + RS (30 mg/kg)	26±0.36[b]
HFD + RS (60 mg/kg)	29±0.64[b]

Each group (n=6) represent mean ± standard errors of means (S.E.M)

a= $p< 0.05$ Vs. Transfer latency on first day of control group.

b= $p< 0.05$ Vs. Transfer latency on first day of control group.

Effect of HFD, STZ and *Rauwolfia serpentina* in brain Thiobarbituric acid Reactive Species (TBARS) levels

HFD and STZ control group produced significant ($p<0.01$) increase in brain oxidative stress level as determined by increase brain Thiobarbituric acid reactive species (TBARS) level when compared to control group. However treatment with *Rauwolfia serpentina* (10, 30, 60 mg/kg p.o) significantly ($p<0.01$) inhibited the HFD and STZ induced rise in brain oxidative stress levels as compared to HFD control group. In contrast, treatment with Atorvastatin and Vitamin E also produced significant ($p<0.01$)($p<0.05$) effect on HFD and STZ induced rise in brain oxidative stress levels as compared to HFD and STZ group (Table 5.4).

Table 5.4: Effect of HFD, STZ and Rauwolfia serpentina (RS) in brain Thiobarbituric acid reactive species (TBARS) levels.

Group	TBARS(nmol/mg of protein)
Control	6.2±0.92
STZ	18±1.94[a]
STZ + Vitamin E	11.8±1.44[b]
STZ + RS (10mg/kg)	12.5±0.92[b]
STZ + RS (30 mg/kg)	12.0±0.96[b]
STZ + RS (60 mg/kg)	12.3±0.44[b]
HFD	17±0.95[a]
HFD + Atorvastatin	19±1.68[b]
HFD + RS (10mg/kg)	12±0.94[b]
HFD + RS (30 mg/kg)	11.2±0.66[b]
HFD + RS (60 mg/kg)	7.8±0.54[b]

Each group (n=6) represent mean ± standard errors of mean (S.E.M)

a= $p< 0.01$ vs control group.

b= $p< 0.01$ vs STZ control group.

b= $p< 0.01$ vs HFD control group.

Effect of HFD, STZ and *Rauwolfia serpentina* on Reduced Glutathione Level (GSH) in brain

HFD and STZ control group produced significant ($p<0.01$) increase in brain oxidative stress levels, as determined by reduced Glutathione (GSH) levels in brain when compared to control group. However treatment with *Rauwolfia serpentina* (10, 30, 60 mg/kg p.o) significantly ($p<0.01$) inhibited the HFD induced rise in brain oxidative stress level as compared to HFD to control group. In contrast, treatment with Atorvastatin also produced significant ($p<0.01$) effect on HFD induced rise in brain oxidative stress level as compared to HFD control group (Table 5.5).

Table 5.5: Effect of STZ, HFD and Rauwolfia serpentina (RS) on Reduced Glutathione reductase (GSH) Level in brain.

Group	GSH levels (nmol/mg of protein)
Control	22.4±0.92
STZ	10.9±1.32[a]
STZ + Vitamin E	17.8±0.94[b]
STZ + RS (10mg/kg)	16.4±0.94[b]
STZ + RS (30 mg/kg)	16.2±0.81[b]
STZ + RS (60 mg/kg)	16.8±0.92[b]
HFD	11.5±1.25[a]
HFD + Atorvastatin	15.8±0.98[b]
HFD + RS (10mg/kg)	15.4±0.91[b]
HFD + RS (30 mg/kg)	15.1±0.62[b]
HFD + RS (60 mg/kg)	15.3±0.64[b]

Each group (n=6) represent mean ± standard errors of mean (S.E.M)

a= $p< 0.01$ vs control group.

b= $p< 0.01$ vs HFD control group.

b= $p< 0.01$ vs STZ control group.

Effects of HFD and *Rauwolfia serpentina* on Total serum cholesterol levels

Mice subjected to high fat diet (HFD) for 90 days showed significantly increase in serum cholesterol level compared to control group. However treatment with *Rauwolfia serpentina* (10, 30, 60 mg/kg p.o) and Atorvastatin significantly ($p<0.01$) attenuated HFD induced rise in total serum cholesterol levels (Table 5.6).

Table 5.6: Effect of HFD and Rauwolfia serpentina (RS) on total serum Cholesterol levels.

Group	Day 90	Day 110
Control	110±0.96[a]	117±0.93[a]
HFD	169±1.56[b]	177±1.54[b]
HFD + Atorvastatin	158±0.92[b]	79±0.92[b]
HFD + RS (10mg/kg)	162±1.21[b]	94±1.27[b]
HFD + RS(30 mg/kg)	142±0.98[b]	73±0.98[b]
HFD + RS(60mg/kg)	153±0.94[b]	109±0.91[b]

Each group (n=6) represent mean ± standard errors of mean (S.E.M)
a= $p< 0.01$ vs control group.
b= $p< 0.01$ vs HFD control group.

Effects of HFD on Body weight of mice (gm) ± S.E.M

There was significant ($p<0.05$) increase in body weight of animals over period of 90 days in mice receiving normal diet or high fat diet, when compared to the body weight of mice on day1. Furthermore, HFD treatment for 90 days produced significant($p<0.05$) increase in body weight of mice as compared to those receiving normal diet for 90 days (Table 5.7).

Table 5.7: Effect of HFD on Body Weight of Mice (gm) ± S.E.M.

Group	Day 1	Day 30	Day 60	Day 90
Control (Normal diet)	21±0.96	22±0.94	23±1.24	25±0.92
HFD	23±0.99[a]	25±0.91[a]	26±1.22[a]	28±1.23[a]

Each group (n=6) represent mean ± standard error of means (S.E.M)
a= $p< 0.05$ vs day 1, day 30, day 60 and day 90 body weight in control group.

DISCUSSION

Morris Water Maze test employed in present study is one of the most accepted models to study the evaluation of learning and memory in animals.[23,25] A significant decrease in day 4 escapes latency time (ELT) of control animals during ongoing acquisition trials demonstrated normal acquisition of memory and an increase in time spent in target quadrant(TSTQ) in search of missing platform during retrieval trial indicated, the retrieval of memory.

Elevated plus Maze test is very useful to investigate the antianxiety activity and has been also helpful to measure the cognitive performance, basically to evaluate the spatial long term memory (LTM) in rodents. Transfer latency (TL) of the first day is directly associated with acquisition of information and memory whereas transfer latency (TL) of second day mainly reflects retention of learning and memory.[25]

The STZ i.c.v model has been described as one of an appropriate animal model of dementia which is closely related to Alzheimer's disease, typically characterized) by progressive impairment of learning abilities and memory capacities.[23,24,26] This observation is in line with various previous reports which state that the intracerebroventricular administration of streptozotocin at sub- diabetogenic dose has been shown to impairs memory functions along with increase in brain oxidative stress levels.

Studies have documented that administration of cholesterol rich diet imparts memory deficits in rodents. Increased brain cholesterol has also been documented to raise the ß amyloid peptide, PGE_2 Production, activation of NF-Kß in brain eventually culminating in neuronal damage and dementia.[25]

With above discussed studies, STZ and HFD treated mice in the study performed badly in Morris Water Maze test indicating impairment in their learning abilities and memory capabilities. STZ and HFD induced impairment of acquisition and retrieval of memory which is seen by significant increase in day 4 ELT and decrease in day 5 TSTQ respectively. Intracerebroventricular streptozotocin (STZ ICV) in the study has also impaired learning and memory along with significant rise in brain oxidative stress level. Further there was a significant rise in brain oxidative stress levels (indicated by an increase in TBARS and decrease in GSH levels). The results of present investigation indicate that intracerebroventricular (i.c.v) administration of streptozotocin (STZ, 3mg/kg) has produced significant cognitive deficits, abnormal biochemical alteration in the mice brain similar to that happens in dementia of AD type. High fat Diet (HFD) for 90 days also accentuated the body weight and serum cholesterol levels in a significant manner.

Medicinal herbs are indispensible part of traditional medicine and in the recent past, grab attention due to their potential role in dementia. Polyphenols constitute a large and major group of naturally occurring substances in the plant kingdom, which mainly include the flavanoids. Several epidemiological studies suggest that incorporation of antioxidant rich foods in diet is beneficial in improving cognitive performance in

humans.[26] Furthermore dietary intake of flavanoids has been associated with inversely related to risk of dementia.[23,14] The increasing realization that polyphenols rich diet may directly benefit human health, fuels continued research interest in these compounds.

Rauwolfia serpentina elicit antioxidant capacity due to observed higher phenolic contents like p-hydroxybenzoic acid and p- coumaric acid, flavanol and Proanthocyanidins (flavanoids), gallic acid and Digallic acid. The methanol root extract of *Rauwolfia serpentina* provide 233 mg/gm phenol.[27] Phenolic compounds contribute to antioxidant action due to mechanism like their property of redox reaction, power to chelate metals; they can quench singlet oxygen thereby decreasing chance for reactive oxygen species formation. A highly significant positive correlation which was found between antioxidant capacity and phenolic content, indicate that phenolic compounds are major contributor to antioxidant activity.

In present study, administration of *Rauwolfia serpentina* significantly attenuated HFD induced rise in ELT and HFD induced decrease in time spent in target quadrant TSTQ in MWM; indicating reversal of HFD induced learning and memory deficits. Further administration of *Rauwolfia serpentina* significantly attenuated STZ induced rise in ELT and STZ induced decrease in TSTQ in MWM, indicating reversal of STZ induced learning and memory deficits. It showed significant attenuation of i.c.v STZ and HFD induced increase in brain oxidative stress (enhance TBARS and decrease in GSH levels) which may attribute to its antioxidant activity. HFD treated mice also produced a significant reversal of elevated serum cholesterol levels and memory impairment, which mainly attribute to its cholesterol lowering property.

Hence it is hypothesized that presence of these phytoconstituents and plethora of pharmacological effects particularly antioxidant, antihypergylcemic and anti dyslipidemic effect is suggestive of its potential memory preserving as well as restorative activity.

These finding in the present study, demonstrate the Potential of *Rauwolfia serpentina* extract rich in polyphenolic compound as a safe and effective indigenous drug in memory dysfunction.

CONCLUSION

From above discussion and results it can be concluded that: Treatment with *Rauwolfia serpentina* (10, 30, 60 mg/kg p.o) significantly reversed i.c.v STZ and HFD induced learning and memory deficits. It showed significant attenuation of i.c.v STZ and HFD induced rise in brain oxidative stress (increase TBARS and decrease GSH levels) which may attribute to its anti-oxidative property. The Neuroprotective property of *Rauwolfia serpentina* can be attributed to the phenolic compound like phenolic acid, flavanoids and tannins. HFD treated mice also produced significant reversal of serum cholesterol levels and memory impairment which mainly attribute to its cholesterol lowering effect. The present study also supports an important concept that onset of neurodegenerative disease may be delayed or slows with use of dietary polyphenols that protects against oxidative stress and neurodegeneration.

ACKNOWLEDGMENT

Authors would like to Thank Dr (Prof). Preeti kothiyal and Dr. Arun Kumar for their guidance and moral support during research work.

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