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Original Article

PHARMACOLOGICAL EVALUATION OF TRIPHALA CHURNA INSTREPTOZOTOCIN (I. C. V.) INDUCED DEMENTIA IN RATS

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ABSTRACT

Objective: The objective of the study was to investigate the memory improving activity of Triphala Churna hydro-methanolic fruit extract on learning and memory functions in Streptozotocin (I. C. V) induced dementia in rats by using morris water maze and elevated plus maze.

Methods: A total of 42 albino wistar rats weighing 80-100 g were randomized into 7 equal groups as follows: Normal control group received normal saline (1 ml/kg p. o.) for 24 d, STZ treated group (3 mg/kg, i. c. v) were administered in two dosage regimen i.e. on first day and third day.), Standard group: Streptozotocin (3 mg/kg i. c. v)+Vitamin E (100 mg/kg/day p. o.) were administered for 21 d, Standard group: Streptozotocin (3 mg/kg i. c. v)+Rivastigmine (2 mg/kg/day p. o.) were administered for 21 d. The learning and memory-impaired rats were treated with Triphala Churna Formulation 1, Triphala Churna Formulation 2 and Triphala Churna Formulation 3 for 21 d (100 mg/kg p. o.). AchE activity, lipid peroxidation, superoxide dismutase, glutathione level of brain homogenate was estimated in Control/STZ (I. C. V)/Standard/Triphala Churna fruits extract treated rats.

Results: Administration of Triphala Churna fruits extract significantly restored learning and memory impairment induced by STZ (I. C. V) in the elevated plus maze and morris water maze. Furthermore, in the TPLC F2 and TPLC F3 treated group brain AchE level was decreased (P≤0.01) as well as brain lipid peroxidation was also decreased (P≤0.001). Brain antioxidant enzymes such as glutathione level were increased (P≤0.001) in the TPLC1 and TPLC2 treated group when compared to the STZ treated group, TPLC F2 and TPLC F3 treated group showed significant (P≤0.001, P≤0.01) increase in superoxide dismutase level.

Conclusion: Triphala Churna fruits extract has an improving effect on learning and memory impairment rats produced by Streptozotocin (I. C. V) and may have a useful effect in the treatment of dementia and Alzheimer's disease.

Keywords: Triphala Churna, AchE, Vitamin E, Rivastigmine, Streptozotocin (I. C. V.) and antioxidant

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INTRODUCTION

Acquisition of information and skill is defined as learning [1]. Through two processes behaviour is modified by experience–one is learning which means the acquisition of new knowledge about the environment and another is a memory which means its retention [2]. Dementia is a brain disorder denoted by a decrease in several higher mental functions such as memory, intellect, a personality that occurs serious impairments in daily life [3]. The prevalence of dementia increases with age, doubling every 5 y between the ages of 60 and 90 [4]. Dementia is induced by a disease that impairs tissues in the brain causing the destruction of brain functioning. Dementia is characterized by irreversible and reversible causes. Dementia due to long-term substance abuse, accumulation of blood beneath the outer covering of the brain that result of head injury, subdural hematoma, normal pressure hydrocephalus, toxic reactions like excessive alcohol or drug use, hypothyroidism, and nutritional deficiencies like vitamin B12 and folate deficiencies. Some of the irreversible and non-treatable cause of dementia involves diseases that cause degradation or loss of nerve cells in the brain such as PD, AD [4, 5] and HD, multi-infracts dementia (dementia because of multiple small strokes, also known as vascular dementia), infections that affect the spinal cord and brain, for example acquired-immune deficiency syndrome (AIDS) dementia complex and Creutzfeldt-Jakob disease. Some people have a combined type of dementia including both vascular dementia and AD. The most common symptoms that are mainly associated with dementia are psychosis, delirium from a sudden medical problem, aggression, insomnia or —sundowning (confusion in the early evening or late afternoon), anger, anxiety, pain from arthritis and depression [4, 6]. It represents a decrease in the previous level of functioning and it is also associated with impairment in psychiatric disturbances, behavioural and functional abilities [7, 8].

Intracerebroventricular (ICV) administration of streptozotocin (STZ) in rats is frequently employed to study experimental dementia by understanding some of the pathological aspects of SAD in humans, currently, [26]. Moreover, it has been well expressed that ICV-STZ rat model is targeting the functioning of brain IR signalling cascade. Decreased levels of glucose/energy metabolism especially in cerebral cortex and hippocampus areas in the brain have been investigated starting from 3 w following ICV-STZ administration and hence mitochondrial dysfunction [14]. Further, a progressive trend towards oxidative stress has also been obtained starting as early as 1 w following the ICV-STZ administration [27]. In addition to decreased energy metabolism and mitochondrial dysfunction, increased free radical generation, subsequent oxidative as well as nitrosative stress which are well revealed to impair learning and memory leading to cognitive dysfunction [28].

In ayurveda, triphala is well known as polyherbal formulation. In Indian system of medicine, it is used as a rasnayana drug [9]. Triphala is consists of the three dried fruits of trees and they are Indian gooseberry (*Emblica officinalis* Gaertn, family *Euphobiaceae*), Belleric myrobalan (*Terminalia belerica*Linn. Family, *Combretaceae*) and Chebulic myrobalan (*Terminalia chebula* Retzr. Family *Combretaceae)* [10, 11]. It is composed equal proportion (1:1:1) of these three fruits as described in ayurvedic formulary of india. Triphala is termed as tridosic rasnayana in ayurveda and it is having rejuvenating and balancing effect on three constitutional elements which regulate the human life (vata, pitta and kapha)[9]. The most commonly found polyphenolic compounds in the plant extracts are phenolic acids, flavonoids and tannins. 70 % Methanolic extract of triphala exhibit antioxidative properties by the following order *T. Chebula* > *E. Officinalis* >*T. Bellerica.* In their flavonoid content, the same order is followed, while in case of phenolic content the order is

E. officinalis>*T. belerica*>*T. chebula* [12]. The aim of my study was a pharmacological evaluation of Triphala churna in streptozotocin (i. c. v.) induced dementia in rats.

MATERIALS AND METHODS

Chemicals and reagents

Acetic acid, Acetylthiocholine iodide, Bovine serum albumin, Calcium chloride, Chloral hydrate, Copper sulfate solution, Dextrose, Ethylene diamine tetra acetic acid (EDTA), Folin-ciocalteau reagent solution, Hydro-xylamine hydrochloride, Magnesium Chloride, Methanol, Nitro blue tetrazolium (NBT), Potassium chloride, Potassium phosphate dibasic, Protein estimation kit, Reduced glutathione, Streptozotocin(STZ), Sodium chloride (Nacl), Sodium potassium tartrate, Sodium hydroxide, Sodium dihydrogen phosphate, Sodium Carbonate, Sodium citrate, Superoxide dismutase colorimetric assay kit, Thiobarbituric acid (TBA), Trichloroacetic acid (TCA), Triton X-100 were purchased from Central drug house (P) Ltd New Delhi. Diethyl ether was purchased from SGRRITS; Vitamin E was purchased from Merck Limited, Goa; Rivastigmine was purchased from Sun Pharma Laboratories LTD, Sikkim.

Procurement of triphala churna

Emblica officinalis (Anwala Churna), Terminalia bellerica (Baheda Churna), and Terminalia chebula (Harad churna), manufactured at Vyas Pharmaceuticals, indore-452015 were obtained from the authorized Ayurvedic shop (Krishna Medical Hall, Paltan Bazar), local market, Dehradun, Uttarakhand.

Animals

Albino wistar rats weighing 80-100 g, were procured from animal house facility of Division of pharmaceutical science, Shri Guru Ram Rai Institute of Technology and Science, Patel Nagar, Dehradun. The care of laboratory animals and all the procedures on animals were performed in strict accordance with the CPCSEA, Ministry of forest and environment Government of India.

The protocol was approved by the Institutional Animal Ethics Committee (Registration No. 264/PO/ReBi/S/2002/CPCSEA) and will be carried out in accordance with the CPCSEA guidelines.

Experimental protocol

In present study 7 groups was employed and each group will be comprised of 6 animals.

Group I: Normal control group: Normal saline (1 ml/kg p. o. daily) was administered before evaluation of learning and memory for 24 d.

Group II: Disease induced group: Streptozotocin (3 mg/kg, i. c. v) was administered in two dosage regimen i.e. on first day and third day.

Group III: Standard group: Streptozotocin (3 mg/kg i. c. v)+Vitamin E (100 mg/kg/day p. o.) was administered for 21 d.

Group IV: Standard group: Streptozotocin (3 mg/kg i. c. v)+Rivastigmine (2 mg/kg/day p. o.) was administered for 21 d.

Group V: Streptozotocin (3 mg/kg i. c. v)+Triphala Churna Formulation 1 (100 mg/kg/day p. o.) was administered for 21 d

Group VI: Streptozotocin (3 mg/kg i. c. v)+Triphala Churna Formulation 2 (100 mg/kg/day p. o.) was administered for 21 d.

Group VII: Streptozotocin (3 mg/kg i. c. v)+Triphala Churna Formulation 3 (100 mg/kg/day p. o.) was administered for 21 d.

Extraction method

The powder (100 g) of the individual normal air-dried fruits of *T. chebula*, *T. belerica* and *E. officinalis* was stirred using a magnetic stirrer with a 7:3 mixture of methanol: water (500 ml) for 15 h; the mixture was then centrifuged at 2850 × *g* and the supernatant decanted. The process was repeated by adding the solvent with the precipitated pellet. The supernatants were collected, concentrated in a rotary evaporator [250-200 mbar at 37 °C] and lyophilized. The yields for the plant's materials were 5.2 g, 3.8 g and 4.5 g for *T. chebula*, *T. belerica* and *E. officinalis*, respectively. The dried extracts were stored at-20 °C until use [12].

Three formulations are:

TPL Churna formulation 1 (*T. Chebula: E. Officinalis: T. belerica*)=1:1:1

TPL Churna formulation 2 (*T. Chebula: E. Officinalis: T. belerica*)=2:1:1

TPL Churna formulation 3 (*T. Chebula: E. Officinalis: T. belerica*)=1:2:1

Streptozotocin (ICV STZ)-induced dementia

Rats were anaesthetized with anaesthetic ether and I. C. V. injections were made with a hypodermic needle of 0.4 mm external diameter attached to a 10 µl hamilton microlitre syringe. The needle was covered with a polypropylene tube except for 3 mm of the tip region so as to insert this portion of the needle perpendicularly through the skull into the brain of the rats. STZ was dissolved in freshly made artificial cerebrospinal fluid ACSF (25 mg/ml) solution. The injection site was 1 mm to right or left midpoint on the line drawn through to the anterior base of the ears. Injections were performed into the right or left ventricle randomly. Two doses of STZ (3 mg/kg) were administered by I. C. V. injection bilaterally. The second dose was administered 48 h after the first dose. The concentration was adjusted so as to deliver a maximum of 5 µl in a single injection [13].

Preparation of ACSF for STZ

Artificial Cerebro Spinal Fluid is consists of 147 mmol NaCl; 2.9 mmol KCl; 1.6 mmol $MgCl_2$; 1.7 mmol $CaCl_2$ and 2.2 mmol dextrose [14].

Morris water maze

Acquisition trial

In this study, spatial learning and memory of animals were tested in a morris water maze. The test was performed on day 20-23 and a probe trial was performed on day 24. It is comprised of a circular water tank (180 cm diameter, 60 cm height) filled with water (25±1 °C) to a depth of 40 cm. An escape platform (12.5 cm in diameter and 38 cm high) was put in the pool 2 cm below the surface of the water. The escape platform was settled in the middle of one of the randomly selected quadrants of the pool and kept in the same position throughout the entire experiment. A non-toxic water-dispersible emulsion (milk) was used to render the water opaque. Four equally spaced locations around the edge of the pool (North, South, East, and West) were used as start points, which divided the pool into 4 quadrants. Without a platform, before the training started the rats were allowed to swim freely into the pool for 120s. For each trial, each rat was put into the water at one of four starting positions, the sequence of which being selected randomly. During test trials, rats were placed into the tank at the same starting point, with their heads facing the wall and allowed to locate submerged platform within 120 sec. The rats received four consecutive daily training trials in the following 5 d, with each trial having a ceiling time of 120 s and a trial interval of approximately 30s. The rat had to swim until it climbed onto the platform submerged underneath the water. After climbing onto the platform, the animal remained there for 30s before the commencement of the next trial. If the rat failed to reach the escape platform within the maximally allowed time of 120s, it was guided with the help of a rod and allowed to remain on the platform for 30s. The time taken by rats to locate the hidden platform (latency in seconds) was measured. Animals were subjected to training trials for 5 consecutive days, the starting point was changed with each exposure as mentioned below and target quadrant (Q4 in the present study) remain constant throughout the training period.

Memory consolidation test

Twenty four hours after the acquisition phase, a probe test (day 24) was performed by removing the platform wherein the extent of memory consolidation was assessed. Rats were allowed to swim freely in the pool for 120s. The time spent by rats in target quadrant, which had previously contained the hidden platform, was noted. The time spent by rats in the target quadrant which indicated the degree of memory consolidation which had taken place after learning [15, 16, 5].

Elevated plus maze

An elevated plus-maze was used to test the cognitive function of animals. It is constituted of two opposite open arms (50 cm × 10 cm), crossed with two closed arms of the same dimensions with 40 cm high walls. The arms were connected by a central square (10 cm × 10 cm) and the apparatus was elevated 70 cm from the floor [17]. Memory acquisition and retention were tested using elevated plus maze test on days 23 and 24. Each rat was placed on the open arm, facing outwards. The time taken by the rat to enter the closed arm in the first trial (acquisition trial) on 23th day was noted and was called as initial transfer latency. Cut-off time was fixed at 90s and in case a rat did not move into the closed arm within this period, it was gently pushed into one of the closed arms and for 30 s it was allowed to explore the maze. The second trial (retention trial) was performed 24 h after the acquisition trial and retention transfer latency was noted. The retention trial latency was expressed as a percentage of initial trial latency [18].

Biochemical parameter

Brain collection and preparation of brain homogenate for estimation of AChE activity

The rats were decapitated by cervical dislocation under light anesthesia; brains are removed quickly and placed in ice-cold saline. 50 mg of tissue was homogenized in 0.1M Phosphate buffer which is having pH 8 (5 ml).

Estimation of acetylcholinesterase (AChE) activity

The method of AChE activity estimation is popularly known as Ellman's method, developed by George Ellman in 1961. The brain cholinesterase activity was estimated by providing an artificial chemical, acetylthiocholine (ATC). Thiocholine released because of the cleavage of ATC by AChE is allowed to react with 5, 5-dithio-bis-(2, nitrobenzoic acid) (DTNB), which is reduced to thionitrobenzoic acid, a yellow coloured anion with absorption maxima at 412 nm.

Procedure

Brains were removed quickly and placed in ice-cold saline. 50 mg of tissue was homogenized in 0.1M Phosphate buffer which is having pH 8 (5 ml). To a cuvette containing 2.6 ml phosphate buffer (0.1M, pH 8), 0.4 ml aliquot of the homogenate is added and 100µl of DTNB. Thoroughly the contents of the cuvette were mixed by bubbling air and absorbance is measured at 412 nm in a spectrophotometer. When absorbance obtains a stable value, it is recorded as the basal reading. 20µl of substrate i.e., acetylthiocholine is added and change in absorbance is recorded [19, 20].

Preparation of Brain homogenate for antioxidant enzyme estimation

Brain tissue samples were homogenized in 0.9% NaCl by using tissue homogenizer [21].

Estimation of lipid peroxidation

Lipid peroxidation levels were measured by the Ohkawa *et al.* method. Taken 2 ml of brain homogenate, add 2 ml of 30% of trichloroacetic acid after that add 0.8% thiobarbituric acid reagent in a test tube. The tubes were covered with aluminium foil and kept in shaking water bath for half an hour at 80 °C. After half an hour; the tubes were taken out and kept in ice-cold water for half an hour Keep test tube in cold water for half an hour. Then process homogenate was centrifuged at 3000 RPM for 15 min, the supernatant was separated out and absorbance was noted down at 535 nm against blank [21].

Estimation of Brain glutathione

Reduced glutathione estimation was done by the method of Sedlak and Lindsay, 1968. To 2 ml of 10% of homogenate, 2.5 ml of 0.02M EDTA was added and shaken vigorously. To 2 ml of this mixture, 4 ml of cold distilled water and 1 ml of 50% trichloroacetic acid were added and was shaken for 10 min. Thereafter, the content was centrifuged at 3000 x *g* for 15 min following centrifugation, 2 ml of the supernatant was mixed with 0.4M tris buffer (pH 8.9). The whole solution was mixed well and 0.1 ml of 0.01M DTNB was added, the absorbance was read within 5 min of addition of DTNB at 412 nm against reagent blank with no homogenate. For blank reading, the homogenate was substituted by 2 ml of distilled water (Sedlak and Lindsay, 1968) [21].

Estimation of superoxide dismutase

SOD was estimated according to Kono method 1978. 1.3 ml of solution A, 0.5 ml of solution B and 0.1 ml of solution C, 0.1 ml of solution D were mixed and rate of NBT reduction was recorded for one minute at 560 nm on Schimadzu spectrophotometer UV120-01. 0.1 ml of the supernatant was added to the test and reference cuvettes, which do not contain solution D. Finally, the % inhibition in the rate of reduction of NBT was recorded as described above in U/g tissue. One enzyme unit was expressed as the inverse of the amount of protein (mg) required inhibiting the reduction rate by 50% in one minute. The activity was calculated using the % inhibition in a gram of tissue and expressed in Unit/g tissue [22, 23].

Estimation of total protein

According to the method of Lowry OH, *et al.* 1951 total protein was done. Total amount of brain total protein was expressed in mg [15].

Statistical analysis

The statistical analysis was carried out using prism graph pad 7software. All values were represented as mean±SEM. Multiple comparisons between different groups was performed using one-way analysis of variance followed by Tukey's test for all biochemical evaluation except in morris water maze.

RESULTS

Pharmacological study

Behavioral estimation

Effect of STZ (i. c. v.) on escape latency time (ELT) and time spent in target quadrant (TSTQ) on morris water maze in rats

Control group showed a significant decrease ($p \leq 0.001$) in day 4 ELT when it was compared to its ELT on day 1(fig. 1). In control group, rats spent significantly ($p \leq 0.001$) more time in the target quadrant (Q4) when it compared to the time spent in other quadrants (Q1, Q2, Q3) (fig. 2). STZ treated group showed a significant ($p \leq 0.001$) increase in ELT when it compared to ELT of the control group (fig. 1) on the same day. STZ treated rats also showed significant ($p \leq 0.001$) decrease in time spent in target quadrant (TSTQ) when it compared time spent in target quadrant (Q4) of control group (fig. 2).

Effect of Triphala Churna fruits extract on escape latency of streptozotocin-treated rats in morris water maze (MWM)

Vitamin E and Rivastigmine treated group significantly ($P \leq 0.001$) improve the ELT when compared with STZ treated group. TPLC F2 showed more significant result ($P \leq 0.001$) when compared to the STZ treated group (fig. 3).

Effect of Triphala Churna fruits extract on time spent on targeted quadrant (TSTQ) of streptozotocin-treated rats in morris water maze (MWM)

When we compared among the Control, STZ, STZ+Vitamin E, STZ+Rivastigmine, STZ+TPLC F1, STZ+TPLC F2, STZ+TPLC F3 during time spent in targeted quadrant there is a significance difference. Administration of Vitamin E and Rivastigmine showed high significance at ($P \leq 0.001$) when compared with STZ treated group. TPLC F1, TPLC F2, TPLC F3 (100 mg/kg p. o.) showed high significance at ($P \leq 0.001$) when compared with STZ treated group (fig. 4).

Effect of Triphala Churna fruits extract on transfer latency (TL) of streptozotocin-treated rats in elevated plus maze (EPM)

Treatment with streptozotocin showed significant ($P \leq 0.001$) increase in TL time when compared with control group. Hydro-methanolic extract of TPLC F1, TPLC F2, TPLC F3 (100 mg/kg p. o.) and standard (Vitamin E, Rivastigmine) significantly ($P \leq 0.001$) reduce TL time when compared to STZ treated group. The effect of TPLC F2 (100 mg/kg p. o.) was noticed to be comparable to standard (Vitamin E) treated group (fig. 5).

Biochemical estimation

Effect of Triphala Churana fruits extract on brain AchE activity of streptozotocin (i. c. v.) treated rats

Treatment with streptozotocin showed significant (P≤0.001) increase in brain AchE activity when compared to control group. The rats were treated with Vitamin E and Rivastigmine showed significant (P≤0.001) decrease in AchE activity when compared to streptozotocin treated group, whereas a significant (P≤0.01) decrease in AchE activity was observed in the TPLC F3 and TPLC F2 treated group. TPLC F1 group showed low significance at P≤0.05 while compared to the STZ treated group (fig. 6).

Effect of Triphala Churana fruits extract on lipid peroxidation of streptozotocin (i. c. v.) treated rats

Lipid peroxidation study showed that streptozotocin treated group showed significant (P≤0.001) increase in MDA level when compared to control group. Standard (Vitamin E and Rivastigmine) and TPLC F2, TPLC F3 treated group were able to prevent the elevated MDA level, showed significant at (P≤0.001) and TPLC F1 showed significance at (P≤0.01) decrease in MDA level when compared to streptozotocin treated group (fig. 7).

Effect of Triphala Churna fruits extract on level of glutathione streptozotocin treared rats

Streptozotocine treated group showed significant (P≤0.001) decrease in glutathione level when compared with control group. Whereas, standard (Vitamin E and Rivastigmine) treated showed significant (P≤0.001) increase in activity of glutathione when compared to streptozotocin treated group. In another hand TPLC F1, TPLC F2 treated group showed significant (P≤0.001) increase in glutathione level when compared to STZ group but results are not better than the standard treated group. TPLC F1 showed low significance (P≤0.01) when compared to STZ group (fig. 8).

Effect of Triphala Churna fruits extract on level of SOD streptozotocin-treated rats

Streptozotocin-treated group showed significant (P≤0.001) decrease in SOD level when compared with control group. Whereas, standard (Vitamin E and Rivastigmine) treated showed significant (P≤0.001) increase in activity of SOD level when compared to streptozotocin treated group. In another hand TPLC F2 and TPLC F3 treated group showed significant (P≤0.001, P≤0.01) increase in SOD level when compared to the standard treated group but results are not better than standard treated group (fig. 9).

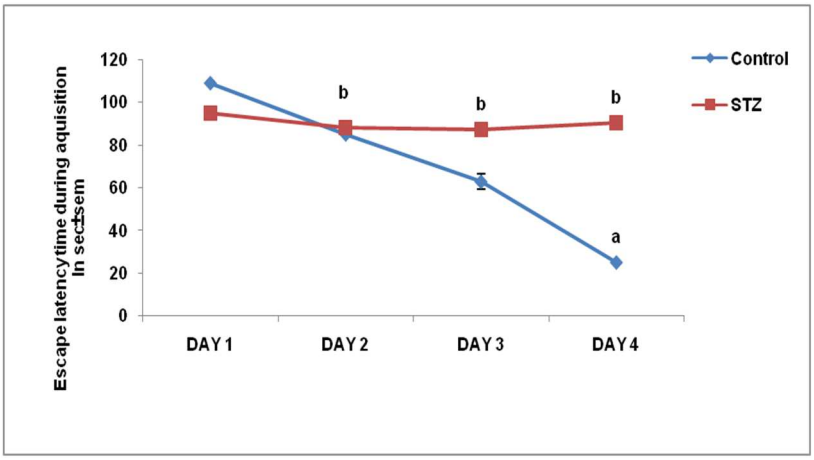

Fig. 1: Effect of STZ (i. c. v) on escape latency time (ELT) during acquisition trials on morris water maze in rats. Each group (n=6) represents mean±standard error of means (SEM), a= p≤ 0.001 Vs. ELT on the first day of the control group, b= p≤ 0.001 Vs. ELT on the same day of the control group. STZ-Streptozotocin

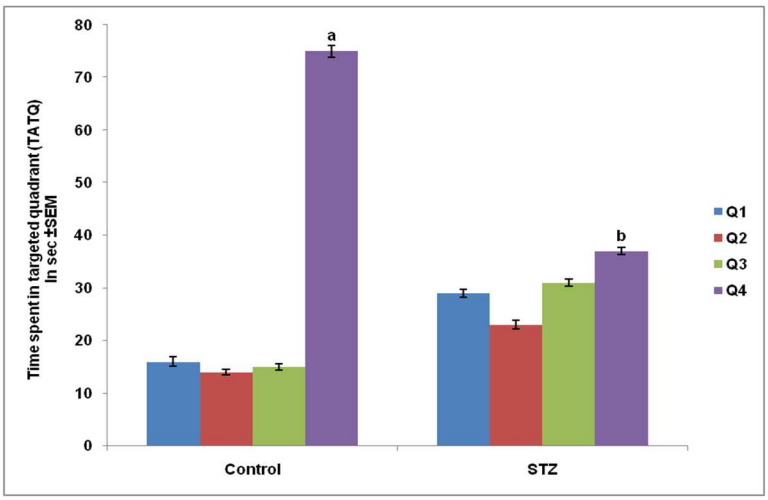

Fig. 2: Effect of STZ (i. c. v) on time spent in the target quadrant (TSTQ) during retrieval trial on morris water maze in rats. Each group (n=6) represents mean±standard error of means (SEM), a= p≤ 0.001 vs. time spent in another quadrant in STZ treated group, b=p≤0.001 vs. time spent in the target quadrant (Q4) of STZ treated group. STZ-Streptozotocin

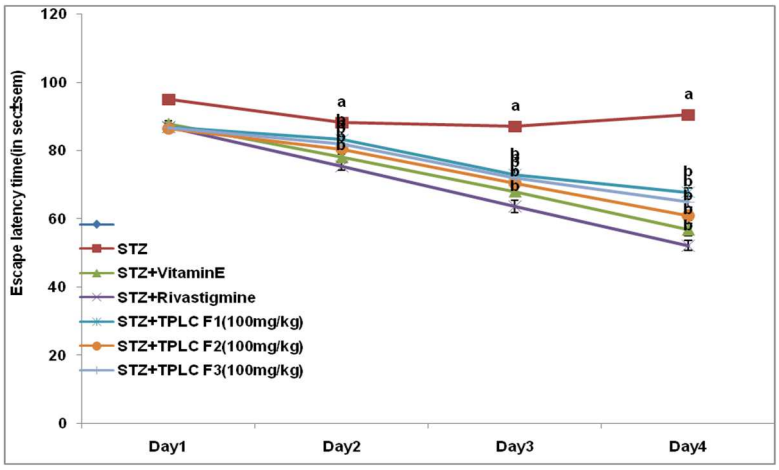

Fig. 3: Effect of Triphala Churna fruits extract on escape latency of streptozotocin-treated rats on morris water maze (1-4 d) in sec. Each group (n=6) represents mean±standard error of means (SEM), a= p≤ 0.001 vs. ELT on the first day of STZ (i. c. v) treated group, b=p≤0.001 vs. ELT of STZ (i. c. v) treated group. STZ-Streptozotocin, TPLC-Triphala Churna, F1-Formulation 1, F2-Formulation 2, F3-Formulation 3

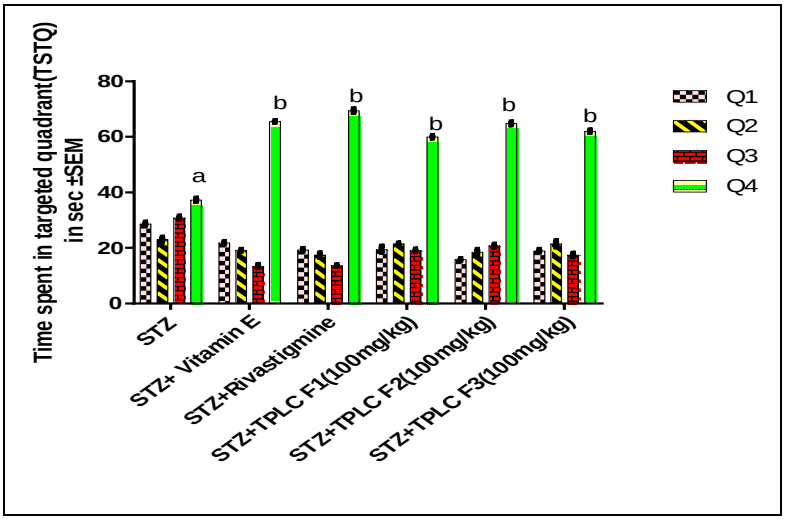

Fig. 4: Effect of Triphala Churna fruits extract on time spent in target quadrant (TSTQ) during retrieval trial in STZ treated rats on morris water maze. Each group (n=6) represents mean±standard error of means (SEM). a= p≤ 0.001 vs. time spent in another quadrant in STZ treated group, b=p≤0.001 vs. time spent in target quadrant (Q4) of STZ treated group. STZ-Streptozotocin, TPLC-Triphala Churna, F1-Formulation 1, F2-Formulation 2, F3-Formulation 3

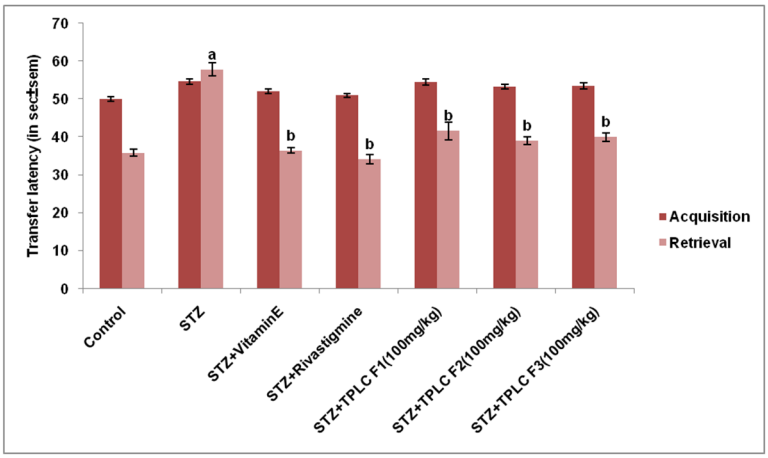

Fig. 5: Effect of Triphala Churna fruits extract on transfer latency (TL) of streptozotocin-treated rats on elevated plus maze (acquisition and retrieval in sec±SEM). Each group (n=6) represents mean±standard error of means (SEM), a= P≤0.001 Vs control group, b=P≤0.001 Vs STZ treated group. STZ-Streptozotocin, TPLC-Triphala Churna, F1-Formulation 1, F2-Formulation 2, F3-Formulation 3

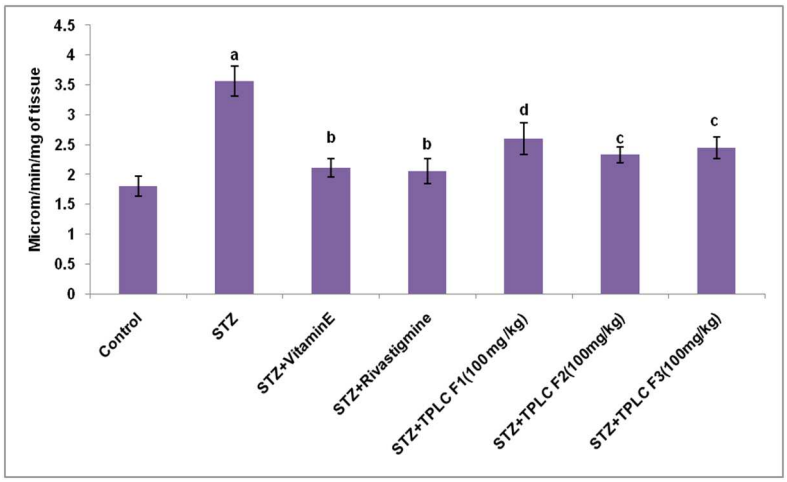

Fig. 6: Effect of Triphala Churna fruits extract on brain cholinesterase activity in streptozotocin-treated rats. Each group (n=6) represents mean±standard error of means (SEM), a= P≤0.001 versus control group, b= P≤0.001 versus STZ treated group, c=P≤0.01 versus STZ treated group, d=P≤0.05 versus STZ treated group. STZ-Streptozotocin, TPLC-Triphala Churna, F1-Formulation 1, F2-Formulation 2, F3-Formulation 3

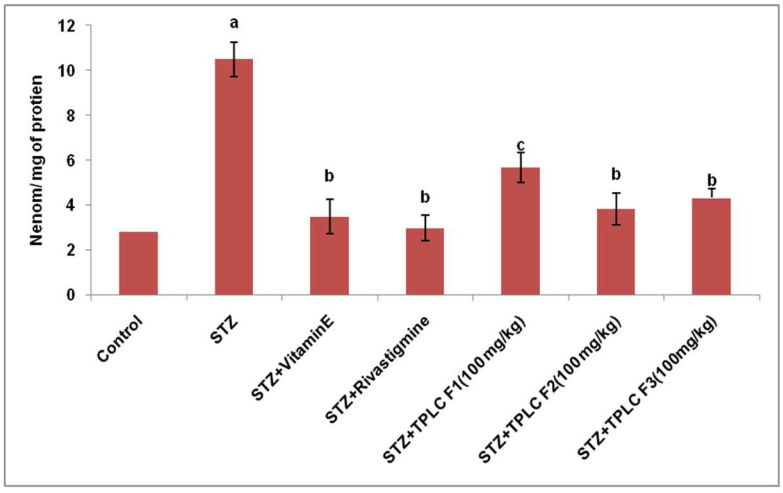

Fig. 7: Effect of Triphala Churna fruits extract on lipid peroxidation in streptozotocin-treated rat's brain. Each group (n=6) represents mean±standard error of means (SEM), a= P≤0.001 versus control group, b=P≤0.001 versus STZ treated group, c=P≤0.01 versus STZ treated group. STZ-Streptozotocin, TPLC-Triphala Churna, F1-Formulation 1, F2-Formulation 2, F3-Formulation 3

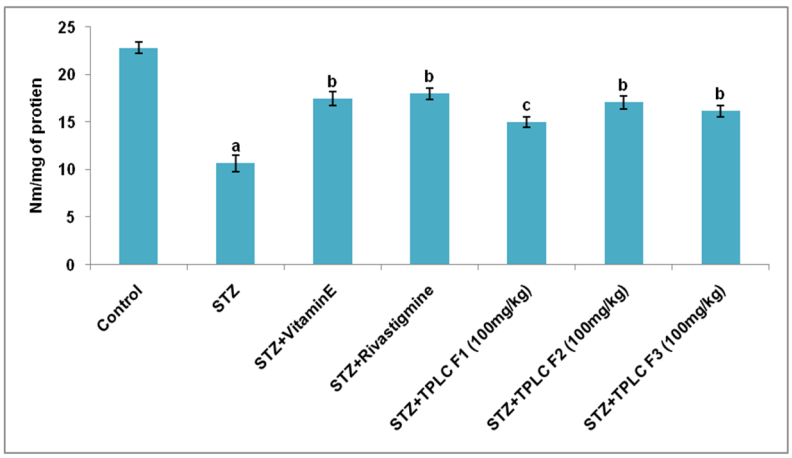

Fig. 8: Effect of Triphala Churna fruits extract on the level of glutathione in streptozotocin-treated rat's brain. Each group (n=6) represents mean±standard error of means (SEM), a=P≤0.001 versus control group, b=P≤0.001 versus STZ treated group, c= P≤0.01 versus STZ treated group. STZ-Streptozotocin, TPLC-Triphala Churna, F1-Formulation 1, F2-Formulation 2, F3-Formulation 3

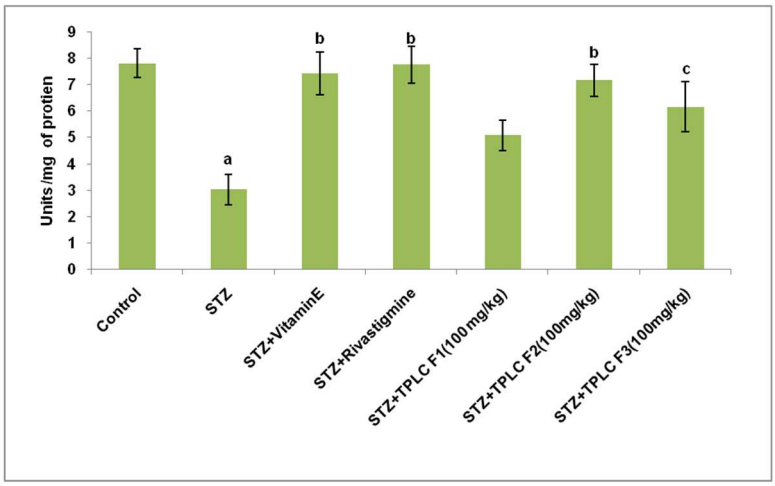

Fig. 9: Effect of Triphala Churna fruits extract on level of superoxide dismutase in streptozotocin-treated rat's brain. Each group (n=6) represents mean±standard error of means (SEM), a=P≤0.001 versus control group, b=P≤0.001 versus STZ treated group, c=P≤0.01 versus STZ treated group. STZ-Streptozotocin, TPLC-Triphala Churna, F1-Formulation 1, F2-Formulation 2, F3-Formulation 3

DISCUSSION

Dementia is an umbrella term that encompasses symptoms of a chronic progressive cognitive decline usually affecting memory and almost always a judgment, decision-making, and relationships with others. Dementia can be divided into cortical and subcortical forms. Alzheimer's disease is a form of cortical dementia, as is creutzfeldt-Jakob disease (CJD). In subcortical dementia, structures below the cerebral cortex are affected or damaged, such as occurs in Parkinson's disease. In multi-infarct dementia both the cortical and subcortical parts of the brain are affected. Dementia can also be divided into reversible causes such as hypothyroidism, normal pressure hydrocephalus, and vitamin B12 deficiency, and irreversible causes such as alzheimer's disease. The most common causes of progressive dementia are alzheimer's disease, vascular dementia, and dementia with lewy bodies, frontotemporal dementia, parkinson 's disease, and wernicke-korsakoff dementia. Alzheimer's disease (AD) is a neurodegenerative disease which involves progressive and irreversible loss of neurons in various regions of the brain. It constitutes approximately 60% of all cases of dementia and is more common in women. It is characterized by impairment of memory and at least one cognitive domain (aphasia, apraxia, agnosia, executive function) [24, 25]. Dementia is one of the characteristics of Alzheimer's disease, Donepezil and Galantamine are the established drugs to treat dementia associated with Alzheimer's disease [33].

Oxidative stress, which occurs when there is an imbalance between antioxidants and reactive oxygen species within a cell, may lead to permanent cellular damage. The polyunsaturated fatty acids of membrane lipids are prime targets for reactive oxygen species and peroxide radicals. The central nervous system is particularly vulnerable to lipid peroxidation because of its high lipid content and unusually high proportion of polyunsaturated fatty acids. In AD patients, concentrations of malondialdehyde, a measure of lipid peroxidation, are elevated. Lipid peroxidation may promote the formation of additional reactive oxygen species and enhance protein and DNA oxidative damage.

Vitamin E has an excellent safety record [32]. Vitamin E is a necessary nutrient in human body and it is a popular antioxidant substance. It decreases reactive oxygen species activity and free radicals like other antioxidants. Vitamin E prevents or delays memory impairments that accompany many conditions such as cerebral ischemic injury, mental stress, aging, diabetes, alzheimer's disease, stroke etc [29].

Rivastigmine is a carbamate-based, reversible, non-competitive inhibitor of both AChE and butyrylcholinesterase. Its inhibitory effect is of long duration and is relatively specific to the central nervous system. Rivastigmine is approved for the mild-to-moderate AD and for mild-to-moderate dementia related to Parkinson's disease [30]. Rivastigmine provides a distinct benefit for AD, and PD, patients with dementia [31].

Streptozotocin significantly impaired the learning and memory, after the administration of streptozotocin on 1^{stand}3rdday. ICV-STZ treatment causes a marked reduction in brain glucose/energy metabolism and shows a progressive trend towards oxidative stress. ICV-STZ generated NMDA receptor overactivation, mitochondrial dysfunction, decrease neuronal glucose which causes a decrease in Ach, ChAT, ATP and increase in AchE etc. These lead to oxidative damage, DNA damage, neuroinflammation after that it causes learning and memory impairment and neurotoxicity.

Morris water maze test and elevated plus maze are employed as most extensively accepted model for evaluation of learning and memory. Rats were taken lesser seconds in transfer latency (retrieval) as compared to transfer latency (acquisition) it means standard and Triphala Churna showed better result in retrieval day. In Morris water maze, after the administration of standard and Triphala Churna significant reduction in escape latency time and also increase time spent in target quadrant on 5th d.

The AchE elevated level in alzheimer's disease has results in the hypothesis that learning and memory impairment is linked to cholinergic degradation. Therefore precise approach for treatment of alzheimer's disease is to increase Ach level in the brain region.

The present study shows that a significant increase in AchE in streptozotocin administered rats. A significant decrease AchE function has been showed in the rats treated with Triphala Churna especially in Triphla Churna Formulation2 (100 mg/kg/day p. o.) dose.

Excessive synthesis of free radicals or the reactive oxygen species can lead to the damage of the cell and tissue of the brain which leads to cell death. The classes of main defense antioxidant that prevent the generation of new free radical species are glutathione and SOD. The further study concluded that the oxidative stress is induced by streptozotocin (i. c. v.) on 1st and ^{3rd}day. In rats streptozotocin decrease the SOD and glutathione level. After the administration of Vitamin E, Rivastigmine and Triphala Churna showed a significant increase in SOD and glutathione level.

An elevated level of lipid peroxidation in the brain shows the neuronal damage. The decrease of antioxidant defense and increase in free radical generation deteriorates the pro-oxidant and antioxidant balance regulation which leads to oxidative stress and cell death.

The oxidative stress produced by streptozotocin has been linked with the increased amount of lipid peroxidation. The administration

of a hydro-methanolic extract of Triphala Churna in my experiment was potentially active in reducing the oxidative stress. This indicates that Triphala Churna fruits extract has a potent antioxidant activity to reduce the oxidative stress induced lipid peroxidation. Triphal Churna Formulation2 (100 mg/kg/d p. o.) showed more improvement in dementia comparison to the other test groups.

CONCLUSION

The aim of the present study was to evaluate the pharmacological effect of Triphala Churna on streptozotocin-induced dementia in rats. This was proved by the following parameters.

The phytochemical constituents in Triphala Churna are phenolic acids, flavonoids, tannins etc. It contains gallic acid, tannic acid, syringic acid, epicatechin, ascorbic acid, aellagitann,interchebulin along with punicalagin, terflavin-A, shikimic, tricontanoic, palmitic acids, beta-sitosterol, daucosterol, triterpene chebupentol etc.

Disease induced STZ (i. c. v.) treated rats shows a significant increase in the AchE activity. Whereas, this increased activity is reverse by treatment of group with Triphala Churna and standard (Vitamin E, Rivastigmine). Reduction in the AchE activity by Triphala Churna fruits extract may directly or indirectly related to the cholinergic dependent learning and memory.

After administration of STZ (i. c. v), disease control rats showed a decrease in free radical scavenging enzymes such as SOD and glutathione. In another hand after the administration of TPLC, free radical scavenging enzyme restored significantly.

In present study treatment by TPLC decreases the lipid peroxidation. Hence, it was concluded the Triphala Churna have a potential role in the management of learning and memory impairment.

ABBREVIATIONS

Acetylcholine (Ach), artificial cerebro spinal fluid(ACSF), alzheimer Disease(AD), adenosine triphosphate (ATP), huntington's disease(HD), parkinson's disease (PD), intra cerebro ventricular(ICV), N-methyl-D-aspartate (NMDA), streptozotocin (STZ), sporadic alzheimer disease(SAD), triphala churna formulation (TPLCF), superoxide dismutase (SOD)

AUTHORS CONTRIBUTIONS

Design, experimental part of the work, analysis of the results, writing and development of the manuscript was done by Purabi Deka. Supervision of the research program was done by Dr. Arun Kumar.

CONFLICT OF INTERESTS

Authors declared no conflict of interests

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