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NEUROPROTECTIVE AND NOOTROPIC EFFECTS OF ETHANOLIC EXTRACT OF *TERMINALIA CHEBULA*

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ABSTRACT

Background: Cerebrovascular accident (CVA) is a world leading cause of mortality and morbidity. Wide modalities of treatment have been used for that and one such modality is neuroprotection. Neuroprotective and Nootropic agents reduces negative consequences of neuronal damage following stroke and scavenges free radicals produced during reperfusion injury, which can even worse out the condition after CVA. **Purpose**: The existing research establishes Neuroprotective and Nootropic effects of T.Chebula against the cerebral ischemic reperfusion injury (acute) and scopolamine induce amnesia in rats so to establish a future treatment modality for injury after stroke and for cognitive disorders. **Methods**: Acute ischemia-reperfusion is induced by blocking of bilateral common carotid arteries for 30 mins thereafter reperfusion for 45 mins in Albino rats(Charles foster). Effects of *T.chebula* on lipid peroxidation, superoxide dismutase (SOD) and T-SH in forebrain regions in acute ischemia - reperfusion were evaluated. Nootropic Effect of *T.Chebula* was evaluated on scopolamine induced amnesia (1mg/kg s.c) via passive avoidance, elevated plus maze and water maze test. **Results**: Pretreatment of rats with *T.Chebula* at a dose of 100 mg/kg p.o for 14 days reversed the biochemical variations produced by reperfusion. Scopolamine persuaded amnesia accompanied by deficits in learning were also attenuated with *T.Chebula* treatment. **Conclusion**: The results imply that *T.chebula* might be useful in neurodegenerative ailments and cerebrovascular insufficiency.

KEYWORDS: *T.chebula*; Cerebral ischemic Reperfusion injury; Oxidative stress; nootropic; Cognition.

1. INTRODUCTION

Neuroprotection is a wide term that refers to all the therapeutic strategies that is used for prevention, treatment and even delay or reversal of any neuronal damage.[1] There are several mechanism that cause neuronal damage and one of the widely accepted mechanism is oxidative stress caused due to any reason.[2]

Oxidative stress occupies an imperative function in the development of human diseases.[3] Disproportion among cellular production of oxygen free radicals and the cell's knack to defend against them via antioxidants is referred to as oxidative stress.[4]

Reactive oxygen species (ROS) production basically produced from enzymatic and nonenzymatic reactions. Now these Enzymatic reactions are those which are engaged in cytochrome P450 system, respiratory chain, phagocytosis and prostaglandin synthesis.[5] Free radicals are highly reactive molecules such as superoxide(O_2), perhydroxy radical (HOO-), hydroxyl radicals(HO), peroxynitrite etc, generates predominantly during cellular respiration and normal metabolism which are formed by one electron deficient process of molecular oxygen (O_2).[6]

ROS mediate peroxidation of lipids which is a chain reaction like event that intensifies the corollaries of the original free radical and precedes to the activation of a sequences of toxic reactions ensuing in gigantic tissue damage.[1] The brain is mainly swayable to lipid peroxide (LPO) since the composition and structure of neuronal tissue makes the brain sensitive to chain reactions facilitated by free radicals and leading to products of LPO.[1] LPO in the brain is one of the key aspects of several neurological and neurodegenerative disorders.[10]

In cerebral ischemia and reperfusion injury these free oxygen radicals play a vital role thus it is one of the

leading causes of oxidative stress.[7] Reentry of oxygen to the ischemic area after restoration of blood supply worsens the damage instead of alleviating ischemic state which is due to ROS production which makes reperfusion injury a dissimilar damage from the primary ischemic injury.[8,9]

Cognitive impairments are a grouping of mental health illnesses that predominantly affects learning, memory, perception, and problem solving, and embrace amnesia, dementia, and delirium. These impairements are progressive in nature and are major global burden in changing age dynamics.[12] A Variety of clinical syndromes like neurodegenerative disorders, traumatic and ischemic brain injury and convulsive disorders manifest as a cognitive dysfunction and memory impairments.[13]

Cognitive defects every now and then display many of the neuropsychiatric illnesses and may sometimes present alone as developmental defects, thus demanding the utility of nootropics to enhance cognitive abilities.[14] Many nootropics have been suggested to provide benefit for those with various ailments of neurodegeneration as: Parkinson's disease, lewy body disease, Alzheimer's disease, Friedreich ataxia, Huntington's disease and dementia.[11,14] Some have even been investigated to help treat the cognitive symptoms of schizophrenia.[15]

Nootropics are also called as memory enhancers, cognitive and intelligent enhancers, smart drug, neuron enhancers. Nootropics are the supplements, drugs, neutraceuticals or functional foods that provide one or more aspects of mental function that are able to promote, enhance and protect cognitive functions where effects can include improvement to working memory, motivation or attention.[11]

"Medhya rasayana",derived from the Sanskrit word, where "Medhya" represents intellect or cognition and "Rasayana" denotes rejuvenation.[16] So these are the cluster of medicinal herbs which is labelled in Ayurveda with the multiple advantages especially to enrich memory retention and intelligance by its prabhava (specific deed).[16] These drugs carry an indispensable role in the treatment of psychiatric and psychosomatic disorders and are utilized for anticipation and treatment of mental disorders of the entire age groups.

Terminalia chebula, a medhya rasayana and a medicinal plant in Ayurveda, is known for its neuroprotective properties since ages.[17] Its traditional hindi name is "harad" in hindi and "hartaki" in Sanskrit.[18] It has various medicinal properties and due to which it is also known as "king of medicine" in Ayurveda.[18] It contains various active phytochemicals in its various segments such as polyphenols, terpenes, anthocyanins, flavonoids, alkaloids, and glycosides, with tannins as its major component consisting of approximately 32%-34%.[19] It contains good amount of phenolic and flavonoid content which is known for its antioxidants properties.[20] It also contains sufficient amount of ascorbic acid.[21,18] It carries countless pharmacological properties some of which are as antioxidant, anticholinesterase, anti carcinogenic, antimutagenic, anti inflammatory and wound healing, cardioprotective, antidiabetic, hepato-reno protective and antimicrobial.[18,22] The phenols have the ability to penetrate the BBB which act as a potential neuro protective agent.[23]

In the present investigation, neuroprotective and nootropic effects of *T.Chebula* was evaluated by producing cerebral oxidative stress and amnesia respectively.

2. METHODS

2.1 Chemicals and reagent

Thiobarbituric acid, nitro blue tetrazolium, 1,1,3,3-tetraethoxypropane and phenazine methosulfate were mainly used.

2.2 Sample Collection

The dried fruit of terminalia chebula was gathered from the regional area of Varanasi and identified, verified in Dravyaguna department, Faculty of Ayurveda, Institute of medical sciences, BHU.

2.2.1 Preparation of extract

The dried fruit of terminalia chebula was washed thoroughly, chopped into small pieces, allowed for drying under shade, milled or pulverized, and extracted using absolute ethanol for 12 h using a Soxhlet apparatus. Then ethanolic extract was vaporized under reduced pressure at 40°c using a rotator vapour evaporator. The resultant extract was dessicated and protected from light for further analysis and experimental studies. Animals received this extract orally, diluted in carboxy methyl cellulose (CMC) at a dosage of 100mg/kg/day. This dose was chosen accordingly with initial pilot study.

2.3 Animals

After approval from institutional ethical committee (Dean/2019/CAEC/1191), this experiment was done on inbred Albino rats (Charles foster) of both the sexes with body weight 250 to 300 grams. Rats were gathered from the institutional Animal House of IMS, BHU, Varanasi. The animals were properly taken care and housed in cages in suitable settings (25°C±1°C temp; 45-55% humidity; 12:12 hour light-dark cycle). They were freely given water with food under proper hygienic conditions. Rats were allowed to acclimatize in laboratory settings for minimum one week beforehand exercising experiments. Rats were handled according to the Ideologies of laboratory animal preservation (NIH publication number #85-23, revised in 1985).

2.4 Study design

2.4.1 For cerebral oxidative stress

2.4.1.1 Grouping of animals

For acute cerebral ischemic reperfusion study the rats were segregated into following groups(n=6):

First group: Sham operated animals where all surgical procedures were undertaken except bilateral common carotid artery occlusion (BCCAO).

Second group: Perse group where extract (T.chebula) was given to sham operated control.

Third group: Reperfusion injury group where BCCAO was done for 30 mins followed by 45 mins of reperfusion.

Fourth group: Treatment drug (T.Chebula) group where T. chebula was given orally as 100mg/kg/day for 14 days before subjecting animals to ischemia reperfusion.

2.4.1.2 METHOD FOR INDUCTION OF CEREBRAL OXIDATIVE STRESS

A procedure known as Bilateral common carotid artery occlusion (BCCAO) was used for initiating cerebral oxidative stress (Ivasky et al. 1989). Intraperitoneal injection of ketamine (100mg/kg) was injected to anesthetize the rats.

Incision at the midline skin of the neck was given to rats and skin was laterally retracted thereby salivary glands and subcutaneous tissues were freed by blunt dissection and was retracted laterally by means of small haemostats after which both common carotid arteries were parted from adjacent vagus nerve in carotid sheath.

Experiment was initiated when rat's brain was made ischemic by blocking bilateral common carotid arteries for thirty minutes. To block BCCA's, the threads were lifted sufficiently for desired period and after that resupply of blood was done for 45 minutes by just freeing the threads.

Proper Body temperature was sustained (37ºC) during the period. Finally, euthenesia was done by decapitation. Whole brain of rats were dissected out, rinsed well in solution and estimated for biochemical findings.

2.4.1.3 BIOCHEMICAL ESTIMATION

2.4.1.3.1 Sampling technique

After decapitation brain was rinsed with 0.9% NaCl (ice cold). A frontoparietal portion of cerebral cortex from both of the hemisphere was parted and immediately homogenized and was checked for biochemical parameter of the oxidant- antioxidant significance i.e. MDA, SOD, T-SH. Results were compared statistically.

2.4.1.3.2 Measurement of lipid peroxidation

MDA (Malondialdehyde) which is a product of Lipid peroxidation (Thiobarbituric acid reactive substance-TBARS) was estimated (Ohkawa and co-workers. 1979).

2.4.1.3.3 Determination of Superoxide Dismutase Activity

Superoxide dismutase was estimated in ischemic areas (Kakkar and co-workers. 1984). The reduce activity of SOD was checked.

2.4.1.3.4 Determination of Total Tissue Sulfhydryl (T-SH) Groups

Total T-SH in brain was evaluated by the process of Sedlack and Lindsay (1968).

2.4.2 For Nootropic activity

2.4.2.1 grouping of animals

For this study the animals were segregated into following groups (n= 6)

First group: Normal controlled group where normal cmc water was given as a vehicle to animals.

Second group: Amnesic group where scopolamine hydrobromide (1mg/kg sc) was directed instantly following learning trial on day 1.

Third group: Treatment group where T.Chebula 100 mg/kg/day was given for 14 days orally to scopolamine treated animals.

2.4.2.2 Amnesia Model (Scopolamine Induced Amnesia)

Amnesia was induced in animals by administration of scopolamine(1mg/kg, s.c). It was given instantly after the learned trial on 1st day in passive avoidance and on elevated plus maze experiment, whereas after 1st session trial of escape latency in water maze experiment.

2.4.2.3 Behavioural methods

Nootropic activity was evaluated by set of these following experiments.

The retention in Elevated Plus Maze Test (Itoh et al. 1990)

Passive Avoidance Test (Jaiswal and Bhattacharya, 1996)

Morris Water Maze Test (Morris, 1984)

2.4.2.3.1 Transfer latency on elevated plus maze test

The following experiment was exercised to evaluate the preservation of learning and retention of memory (itoh et al., 1990). The elevated plus apparatus was comprised of two opposite open arms (50x10cm) which were intersected by two enclosed arms of same dimensions and walls 40 cm high. The arms was attached with a central square (10x10cm) (gives '+' appearance). Apparatus was kept 50 cm above the floor in a low lit room.

On 1st day, rats were individually positioned on the extreme end of one of the open arms facing away from the center. Time taken by the animal to enter one of the closed arm was noted. This was transfer latency day 1. The rat was leftover in the enclosed arm for a period of 10-15 sec and taken back to its home cage. Transfer latency for all the rats on day 1 was recorded.

On day 2, procedure was recapped and transfer latency on day 2 was recorded.

On day 9, after a break of 1 week, same practice was imitated and transfer latency day 9 was recorded.

The retention scores were acquired for each animal by evaluating percent decrease in latency period from the below mentioned formula:
% Decrease in transfer latency = L1 – L0/ L0 ×100

Where,
L0 = Initial transfer latency period in seconds and
L1= Transfer latency after 24 hours or one week in seconds.

2.4.2.3.2 **passive avoidance test: (step through)**
This experiment used regular performance and behavior of animals. It was established by Kings and Glasser (1970). The following test was assessed by using light dark equipment, which was sectioned into two equal compartments (30x25x30 cm), one of which is light compartment and the next section is a dark compartment. Two walls of this apparatus is crafted up of wood and other two walls of transparent plexiglass. The two equal compartments were divided by plexi glass with a 10x10 cm opening in the epicenter and the opening was controlled by guillotine door. The light compartment was white in color which was illuminated by a 15 w lamp. The dark compartment was black in color. Each compartment had a copper grid floor. To make sure that there was electrical separation, a 1.5 cm gap was created between the two floors, at the entrance in between the two chambers.

On 1st day, rats were located in the light colored box and the time grabbed to move into the dark box was noticed The moment rat enters into the dark compartment, the guillotine box was shut and foot electric shock was delivered for 3 sec of 0.5mA. The Rats were taken back to their cage after the experiment.

On 2nd day, after 24 hour of retention interval, rats were again located into white compartment and was allowed to give a 5 min inhibition period time. Electric shock was not provided on this day. Latency period to step through the dark compartment was recorded. If the animal persisted in the white compartment for a test period of 5 min, a maximum score of 300 sec is allotted.

On day 9th, after a breach of 1 week, latency period to step through was again observed to assess the retention of the passive avoidance learning.

For each animal the retention scores were gained by calculating the "Inflexion Ratio" as follow:
Inflexion Ratio = (L1-L0) / L0
Where,
L0 = Initial step through latency in seconds and
L1 = Step through latency after 24 hours or one week in seconds

2.4.2.3.3 **Morris water maze test (Morris, 1984)**
Memory and Spatial learning was tested in Morris water maze test. The apparatus entailed of a black round pool of 2.14 m diameter and a height of 80 cm. Water at the temp of 25°c was filled upto a depth of 44 cm and was made obscure with Indian ink.

On previous day the rats received habitual trial i.e contact with water in water maze aparatus for a minute where platform does not exist.

On 1st day, a spherical (9 cm diameter) platform was hidden 2 cm beneath the water level in the epicenter of one of the four quadrants. The platform stayed to the same spot at the time of training for reference memory. Now, At the initiation of each term, a random series of four starting poles was engendered along the boundary of the pool. Each of the rats followed this same sequence for this term. Every time rats were submerged at the initial/starting location in water facing the boundary and were allowed to swim for 90 sec to findout the hidden platform. The latency period to find the platform was recorded. However when the rats were unable to treasure the hidden platform, they were taken out and manually positioned on the platform for a period of 20 sec. This same method was taken for all the four initial locations.

Sessions were conducted twice on 1st day of experiment which consisted of four trials. These sessions were gaped by 4 hours. On next day, one session of four trials was conducted.

Subsequently then, a probe trial (without platform) was directed 4 hours later. Rats were submerged randomly, one by one, in the pool at selected starting point. Swimming path was observed and mapped as well as time spent in a particular quadrant where the platform was set aside, was noticed. After the end of the probe trial experiment, a dark colored platform was positioned in a quadrant other than the previously chosen quadrant. This colored platform was raised 1 cm above the surface of water. Every rat was allowed to trace platform for 4 trials(90 sec each).

The latency period to treasure the platform was noticed and is known as working memory procedure.

2.5 Statistical analysis
Statistics was done by one-way ANOVA along with TUKEY test. p-value <0.05 is statistically significant.

3. RESULTS

3.1 Biochemical results
After blocking of common carotid arteries of both the sides for 30 mins(acute) and there after reperfusion for 45 min, the concentration of superoxide dismutase (SOD) and lipid peroxidation(TBARS) and increased

significantly(p value <0.01) whereas T-SH levels were decreased (p value <0.01) in comparison to sham control group(table 1). Extract of T.Chebula reduced enhanced TBARS level (p value < 0.01) and SOD levels (p value < 0.01)(table 1). Also it significantly prevented the consumption of T-SH (p value < 0.05) in comparison to reperfusion injury group. While there was no noteworthy outcomes of any of these biochemical evaluations in perse group.

Table 1: Effect of Terminalia Chebula (100mg/kg/day) on oxidant antioxidants status during cerebral ischemia reperfusion.

Group	MDA (Nm/mg protein)	SOD (mIU/mg protein)	T-SH (x10^{-5}M/mg protein)
Control	1.95 ± 0.25	427.78 ± 40.20	4.49 ± 0.50
Per se	1.99 ± 0.18	456.63 ± 35.21	4.60 ± 0.62
Reperfusion	4.63 ± 0.29[a]	814.29 ± 52.71[a]	3.16 ± 0.27[a]
Treatment	3.14 ± 0.25[a]	572.52 ± 64.82[a]	4.01 ± 0.20[b]

Data are calculated as mean ± SEM. N=6. Inter group comparison is done from control to per se and reperfusion to treatment. p-value is <0.01(a) and <0.05(b). one way ANOVA along with Tukey test is applied.

3.2 Behavioral results

Scopolamine administered rats (1mg/kg s.c) after learning day 1 induced amnesia. This was evidenced from the attenuated decrease in transfer latency and inflexion ratio (p value <0.05) in comparison to vehicle treated on day 2 in both elevated plus maze(table 2) and passive avoidance (table 3) respectively. T.Chebula treated rats were protected from amnesia which is indicated by increase in transfer latency and inflexion ratio(p value <0.01) in both elevated plus maze test and passive avoidance test respectively.

In water maze test rats traced hidden platform during the session of escape trial, whereas scopolamine induced rats took more time than control to trace out the hidden platform. It was noticed that more time was taken by amnesic rats to find the hidden platform during 2nd and 3rd session (amnesic vs control) (p value <0.01) but not during the 1st session (amnesic vs control) as shown in table 4.

T.Chebula pretreatment prevented this delay in escape latency in 2nd and 3rd session (treatment vs amnesic)(p value <0.01). Whereas the results of new platform session showed that as compared to control, amnesic rats took extended time to treasure the new platform (amnesic vs control) (p value <0.01). T.Chebula treated rats prevented this delay in finding hidden platform (p value<0.01)(table 4).

Table 2: Effect of T.Chebula (100mg/kg/day.14 days) on the amnesia in elevated plus maze.

Groups	Percent Decrease in Transfer Latency	
	After 24 hrs	After 1 week
Vehicle	22.19 ± 3.64	27.17 ± 2.67
SCO	3.98 ± 1.98[a]	13.50 ± 3.94[a]
T.Chebula	35.60 ± 4.35[b]	52.14 ± 2.44[b]

Data are calculated as mean ± SEM. N=6. p-value is <0.05(a) and <0.01(b). one way ANOVA along with Tukey test is applied.

Table 3: Effects of T.Chebula (100mg/kg/day, 14 days) on amnesia in passive avoidance test.

Groups	Inflexion Ratio	
	After 24 hours	After one week
Vehicle	4.16 ± 2.21	3.87 ± 2.34
SCO	2.80 ± 1.15	2.55 ± 1.26[a]
T.Chebula	7.70 ± 3.27[b]	6.65 ± 2.59[b]

Data are calculated as mean ± SEM. N=6. p-value is <0.05(a) and <0.01(b). one way ANOVA along with Tukey test is applied.

Table 4: Effect of T.Chebula (100mg/kg/day, 14 days) on cognition in water maze test.

Group	Sessions of Escape latency (seconds)			New platform trial (Seconds)
	1st session	2nd session	3rd session	
Control	96.42 ± 5.20	59.31 ± 4.22	64.31 ± 3.92	52.16 ± 2.98
SCO induced	102.76 ± 6.83	78.36 ± 4.53[a]	82.61 ± 5.27[a]	63.58 ± 4.49[a]
Treatment	93.97 ± 5.69	62.47 ± 5.30[a]	58.81 ± 3.14[a]	48.16 ± 2.04[a]

Data are calculated as mean ± SEM. N=6. Intergroup comparison is done. p-value is <0.01(a). one way ANOVA along with Tukey test is applied.

4. DISCUSSION

In this experimental study, it is intended to explore the potential valuable effects of *Terminalia Chebula*, a familiar medhyarasayana in Indian system of medicine, on cerebral oxidative stress produced by cerebral ischemia reperfusion injury and amnesia developed by scopolamine in rats. The results of our study established the previous reports of production of free radicals from cerebral post-ischemic reperfusion.[7,22] Biochemical parameters also showed that 30 mins of BCCAO and thereafter 45 mins of reperfusion causes ischemic reperfusion injury.[8,25]

Increased free radicals commences lipid peroxidation which is revealed as increase in levels of TBARS in cerebral reperfusion injury.[7,26] After cerebral ischemia leucocytes accumulation occurs and these activated neutrophils generate free radicals especially superoxide anion.[7,24] This increase in SOD activity indicates that brain's antioxidant system is well stimulated in retort to over production of free radicals where SOD activity catalyzes the conversion of superoxide anion to hydrogen peroxide and molecular oxygen.[27,28] Hydrogen peroxide, which got produced by the above reaction, is even more dangerous than the oxygen derived free radicals hence entails to be removed further by tissue thiols (glutathione redox pathway) and catalase,[27,29] There is a decline in GSH levels, during ischemic-reperfusion injury and diminished level of T-SH signifies utilization of tissue thiols which is well reported previously,[7,29] Sulfhydryl compounds are one of the central endogenous antioxidants that works a vital role in maintenance of cellular proteins and lipids in their optimal and functional states. When these are usedup, which is reflected as low levels of T-SH, the deadly effects of oxidative insult are increased causing an increased membrane and cellular damage.[31]

The brain is prone to extreme oxidative stress as it demands high energy and has abundant lipid content.[32] Since ROS buildups proneness to neuronal damage via oxidative changes in the brain, antioxidant therapeutics portray an important role in its protection.[32] The proneness of the brain to oxidative stress is due to limited regenerative capacity, modest antioxidant defenses, peroxidation vulnerability of polyunsaturated fatty acids, the dependence on excitotoxic neurotransmitters, redox-active metal burden, glymphatic waste disposal and calcium load.[33] Oxidative stress prompts several molecular and cellular pathways leading to the steady shortfall of neuronal structures and functions which is considered as a major factor in the development of neurodegenerative ailments like Alzheimer's disease, lewy's disease, Parkinson's disease, Multiple sclerosis, Huntington's disease and Amyotrophic lateral sclerosis.[33] Neuroprotection is a treatment modality for various CNS disorders including stroke, trauma and neurodegenerative progression.[34] The most significant pathological mechanisms in the course of ischemia-induced brain damage include blood-brain barrier (BBB) disruption, inflammatory reaction, oxidative stress and apoptosis of neurons, all of which have been extensively pondered to be the four key therapeutic targets for acute cerebral insult.[34] One of the target for Neuroprotective methods is oxidative stress and excitotoxicity, as excitotoxicity and oxidative stress may lead to neuronal death, hence exacerbation of neuronal degeneration.[34] This justifies the reason behind usage of antioxidants and glutamate antagonists in research and therapies.[34] One of the supplementary benefit of Antioxidants is to block or delay apoptosis.[34] ROS and RNS are also involved in secondary brain damage by injuring membrane lipids, proteins and DNA.[34] Brain has a relatively minimal level of antioxidants, non-replicating character of neuronal cells, little repair capacity and a elevated ratio of membrane surface to cytoplasm, and hence shows the value of targeting antioxidant systems to offset the oxidative stress and associated brain disorders.[32] Antioxidant compounds have been weighed in a number of clinical and preclinical trials as a therapeutic strategy for battling oxidative stress linked with neurodegenerative and other diseases.[32]

The present data tells that *Terminalia chebula* could antagonizes ischemic reperfusion injury induced cerebral oxidative stress which is revealed by reversal of TBARS, SOD, T-SH levels. *Terminalia* treated rats displayed decrease in TBARS and SOD levels and increase in T-SH levels which is in concord with previous reported antioxidant properties of *T.chebula*.[23] Polyphenols, which is found is sufficient quantity in *T.chebula*, have been conveyed to exert their neuroprotective actions by shielding neurons from any damage induced by neurotoxins. Moreover Polyphenols have capability to suppress neuroinflammation, and the great potential to promote skills, memory, learning, and cognitive functions.[34] phenolic contents of *T.chubula*, hence makes it a good antioxidant.[33] Moreover, these phenolic and flavanoids demonstrate neuroprotective properties via an intensification in neuron viability, good amount of tissue perfusion and cerebral blood flow, and have been shown to lessen apoptosis due to ischemia, amyloidogenic effects and dopaminergic loss.[33] One study validated that *T. chebula* extract can lessen infarct volume, improve the sport ability score, and promote rehabilitation.[35] *Terminalia chebula* thus acts as a neuroprotective agent against oxidative stress by feature of its antioxidant and anti-inflammatory properties.

The present analysis also showed the nootropic effects i.e memory enhancing effects of *T.Chebula* in amnesic rats induced by scopolamine. Animals given with extract of test drug showed significant increase in transfer latency and inflexion ratio as compared to SCO treated group in elevated plus maze task and passive avoidance test respectively. SCO treated rats after learning on day 1 induced amnesia which was evidenced from decrease in Transfer latency and inflexion ratio in comparison to vehicle treated rats on day 2. *T.Chebula* extract treated animals were protected against amnesia.

Scopolamine causes dysregulation of cholinergic activity and causes impairment of memory that is evidenced by learning deficits and acquisition and short term retention of spatial memory task.[36,37] Decrease in acetylcholine release causes learning and memory dysfunction as cholinergic system has an imperative role in learning and memory.[38]

In MWM test SCO induced amnesic animals had deficits of cognition. Amnesic models consistently showed

longer escape latencies telling a defective registration of task (learning) moreover longer new platform trial shows deficit of working memory. Decrease in these parameters by extracts of *T.Chebula* showed improvement in spatial learning and memory in amnesic animals.

Different studies of *T. chebula* have also shown concentration-dependent inhibition of acetylcholinesterase and butyrylcholinesterase enzyme which plays a major role in memory and learning.[19] *T. Chebula*, also known for its active constituents such as tannic acid, flavonoids, PGG, ellagic acid and gallic acid which may be in charge for memory and learning effects.[23] One of the special ability of flavonoids is to activate the Akt signaling pathways and the extracellular signal-regulated kinase that leads to the activation of a transcription factor (cAMP response element binding protein), that upsurges the expression of a number of neurotrophins, which is imperative in long term potentiation (LTP) and long-term memory. Example of one such neurotrophin is BDNF(brain derived neurotrophic factor), which is appreciated in controlling synapse growth, strengthening dendritic spine and synaptic receptor density.[34] Gallic acid, a potent antioxidant, is also known for its neuroprotective property.[39] One study showed that *T. chebula* extracts and its components have AChEI, anti-inflammatory effects and antioxidant properties all of which are currently applicable to the treatment of Alzheimer's disease and showed its effectiveness against the progression of AD.[40]

Teminalia chebula as described in Ayurveda is a medhyarasayana i.e memory enhancer drug.[41] Medhyarasyana, also known for its neuroprotection action, plays an imperative role in the treatment of psychosomatic and psychotic diseases by correcting the disturbances of "Rajas" and "Tamas".[42] The manner of this healing involves the individual to achieve calmness, sedation, tranquility or a stimulation of activities of brain.[42] They produce Neuronutrient effect by improving cerebral metabolism. They act through various mechanism such as Neuritic regeneration, synaptic reconstruction, synaptogenesis, axon and dendrite extension. Their effect on cholinergic system that causes decrease in AChE improves memory and learning.[42]

The present study thus reports that *T.Chebula* have neuroprotective role in acute cerebral ischemia reperfusion injury through the virtue of its antioxidant properties and nootropic effects on amnesia.

5. CONCLUSION

Terminalia chebula, as demonstrated in the current study, prevents oxidative stress during cerebral ischemic reperfusion injury and enhanced working and learning memory in scopolamine induced amnesia concluding that *Terminalia Chebula* is a neuroprotective agent by virtue of its antioxidant properties and nootropic agent. Hence *T.Chebula* might be a helpful and better product for cerebrovascular insufficiency state and neurodegenerative disorders like alzheimer's disease and others in future.

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