Original Research Article

Antiamnesic Activity of *Holarrhena antidysenterica* Extract in Streptozotocin-Induced Memory Deficient Rats

Mrinal, Navjeet Singh, Nitin Bansal*
Department of Pharmacology, ASBASJSM College of Pharmacy, Bela (Ropar)-140111, Punjab, India

*Corresponding author
Dr. Nitin Bansal
Email: nitindsp@rediffmail.com

Abstract: The present study is an effort to demonstrate the role of *Holarrhena antidysenterica* Linn in the management of STZ-ICV induced dementia in rats. Wistar rats (either sex; 220-250 g) were divided into 7 groups (n=6 to 8). Ethanol extract of seeds of *Holarrhena antidysenterica* Linn (EEHA) was administered daily in 3 doses (100, 200 and 300 mg/kg; *p.o.*) to rats for 28 successive days. Dementia was induced in rats by intra cerebro ventricular (ICV) injection of STZ (3 mg/kg) using stereotaxic apparatus. Learning & memory assessment with the help of Novel object recognition task and Elevated plus maze was carried out. After behavioural evaluation, brain AChE, TBARS and GSH levels were measured. Administration of STZ to rats increased the transfer latency (TL) (p<0.05) in elevated plus maze as compared to normal control group rats and showed almost equal exploration time of the novel and familiar object in retention trial in novel object recognition task indicating induction of dementia. Furthermore, STZ treated rats showed (p<0.05) decrease in GSH and increase in AchE & TBARS levels. Administration of EEHA (200 and 300 mg/kg; *p*.o) (p<0.05) increased the exploration time of the novel object as compared with familiar object during their retention trial in novel object recognition task and decrease TL in elevated plus maze session as compared to STZ group. EEHA treated rats showed an (p<0.05) increase in GSH and decrease in brain AChE and TBARS levels. Thus, *Holarrhena antidysenterica* Linn may prove to be useful remedy for the management of dementia owing to its possible neuroprotective and antioxidant properties.
Keywords: *Holarrhena antidysenterica* , dementia, Intra cerebro ventricular, Streptozotocin, Oxidative stress.

INTRODUCTION

The human brain is a very complex and intricate machine and many factors can interfere with its functioning. With age, time and medical conditions such as atherosclerosis, stroke, high levels of cholesterol, plasma homocysteine, diabetes and genetic factors a person may lose his ability tosolve problems and maintain emotional control, therefore experience personality changes and behavioral problems such as agitation, delusions, hallucinations and cognitive deficits. Such a mental condition which is characterized by impairment of memory and loss of intellectual ability, sufficiently severe to interfere with one's occupational or social activities is termed as dementia [1, 2]. The term 'dementia' refers to memory impairment and loss of other intellectual abilities which interfere with normal daily activities. The prevalence of dementia is increasing worldwide and became a major public issue with the increasing elderly population. It has been well documented that memory and cognitive impairment is associated with both physiological aging and central nervous system pathological conditions, including stroke, Parkinson's disease (PD) and

Alzheimer's disease (AD) etc. Among them Alzheimer disease is most common form of dementia [3].Memory loss, though common, is not the only sign of dementia.

Alzheimer's disease (AD) is a devastating disease that takes away the very essence of a person – their sense of self. AD, the most prevalent form of dementia, accounts for 50-70 percent of dementia and significantly impacts patients, families, caregivers, communities, and society as a whole. Current medical management of AD is ineffectual, with no cure on the horizon [4]. AD was described by Alois Alzheimer's about 100 years ago. Alzheimer described typical clinical characteristics with memory disturbances and the neuropathological picture with plaques and dense fibrils tangles, which are known as the hall markers of the disease [5]. There are various hypotheses for the pathogenesis of AD. The classic neuropathological signs of Alzheimer's disease are amyloid plaques and neurofibrillary tangles. Plaques consist largely of the protein fragment beta-amyloid. This fragment is produced from a "parent" molecule called amyloid precursor protein. Tangles consist of tau, a protein

normally involved inmaintaining the internal structure of the nerve cell. While tau is normally modified by phosphorylation, or the attachment of phosphate molecules, excessive phosphorylation appears to contribute to tangles formation and prevents the protein from carrying out its normal functions. Oxidative stress, or damage to cellular structures by toxic oxygen molecules called free radicals, is also regarded as a pathology characteristic of AD. Individuals with AD typically experience brain inflammation. However both pathologies are the end result of aggregation of proteins: Aβ gets fibrillized and makes amyloid plaques, while tau protein gets aggregated upon hyper phosphorylation, resulting in NFT pathology [6].

Medicinal plants have been known for millennia and are highly esteemed all over the world as a rich source of therapeutic agents for the prevention of diseases and ailments. The demand for plant based medicines, health products, pharmaceuticals, food suppliment, cosmetics etc are increasing in both developing and developed countries, due to the growing recognition that the natural products are non-toxic, have less side effects and easily available at affordable prices [7]. *Holarrhena antidysenterica* Linn(Family Apocynaceae) is one such plant, popularly known as "Indrajav", "Coneru" in English and "Vatsaka" in Sanskrit is a shrub, distributed throughout India upto an altitude of 4,000 ft. In Indian traditional medicine, the plant has been considered a popular remedy for the treatment of dysentery, diarrhea, and intestinal worms [8]. *H. antidysentrica* Linn a deciduous shrubby 9-10 meter high tree found throughout tropical India, Burma, Srilanka, Pakistan, Nepal and Africa and flowers in the months of May-July. Seeds are linear or oblong concave with a long coma, are light brown in colour and shows epigeal germination. This tree is popular for its numerous medicinal properties and seeds and bark of this tree have been used in Ayurveda since long time. The stem bark which is commonly known as "kurchi" in the Indian subcontinent and as 'conessi bark' in Europe is used in traditional ayurvedic medicine to treat dysentery, especially amoebic dysentery. Bark and leaves of *Holarrhena antidysenterica* Linnis used in Ayurveda as an anti-microbial, anti inflammatory and analgesics [9]. Other useful parts used as medicine are root and leaf. The bark and the roots have been found to be an excellent remedy for both acute and chronic dysentery especially in cases where there is excessive blood with mucus and colic pain associated with stools. In addition the plant has been reported to possess antihelminthic, appetizing, antidiarrhoeal and astringent properties. These properties are due to the presence of steroidal alkaloids conaine and aminopregnane types, the principal one being conessine. *Holarrhena antidysenterica* is also a rich source of other steroidal alkaloids such as kurchine, kurchimine, conessidine, holarrimine, conessidine, conkurchicine and regholarrhimine. *Holarrhena antidysenterica* has been reported to be used as an immunodulating agent, larval

growth inhibitor and against malaria and vaginities [10]. In the light of above literature search, it appears that extract of *Holarrhena antidysentrica* Linn might be useful in the management of dementia of Alzheimer's type. However, no sufficient reports in the literature are available regarding memory affecting property of *Holarrhena antidysenterica* Linn to best of our knowledge. Therefore, the present study is an attempt to explore the effect of *Holarrhena antidysentric*a Linn seeds in the management of dementia.

MATERIALS AND METHODS
Preparation of extract
The seeds of *Holarrhena antidysenterica* Linn were shade dried and it was powdered with gridding process by using grinder. Then, coarse powder was separated with sieve and 250 g of powdered seeds were packed in Soxhlet apparatus and defatted with petroleum ether (40-60ºC) for 72 hours. Then, the defatted seeds powder was extracted with ethanol as a solvent in Soxhlet apparatus. The temperature was maintained on an electric heating mantle with thermostat control. Appearance of colorless solvent in the siphon tube was taken as the symbol of completion of extraction. The extract was concentrated to syrupy consistency on water bath. The concentrated extract was then air dried at room temperature and stored in air tight container at 2–8ºC until used [11].

Animals
Adult Wistar rats (either sex), weighing between 200-250 g, were procured from the Disease Free Small Animal House, Lala Lajpat Rai University of Veterinary and Animal Science Hisar. The animals were kept in quarantine section till monitoring of health status of animals and subsequently transferred to the housing area. The animals were acclimatized for seven days to the housing conditions of Central Animal House Facility of ASBASJSM College of Pharmacy, Bela prior to experiments. Animals were housed in polypropylene cages with dust free rice husk as a bedding material and maintained under standard laboratory conditions with controlled temperature (23 ± 2°C), humidity (40 ± 10%) and natural (12 h each) light-dark cycle. The animals were fed with standard rodent pellet diet (Ashirwad Industries, Mohali) and water *ad libitum*. The experiment was carried out between 09:00 and 18:00 h. The care of laboratory animals was done following the guidelines of CPCSEA, Ministry of Forests & Environment, and Government of India.

Drugs and Chemicals
Holarrhena antidysenterica Linn was procured from Rajesh chemicals Co. Mumbai, Streptozotocin from SRL and Donepezil gifted sample from Cipla limited, Mumbai. Chloroform, Petroleum ether (40-60ºC), ethanol, Folin-ciocalteu reagent from Loba Chemie, Thiobrbituric acid, reduced glutathione, trochloroacetic acid from Hi-media.

Intracerebroventricular administration of streptozotocin

ICV injection of STZ was made according to the procedure of Sonukusare [12]. Rats were anaesthetized with chloral hydrate (400 mg/kg; i.p.). The scalp was shaved, cleaned and cut to expose the skull. The head was positioned in a stereotaxic frame and a midline sagittal incision was made in the scalp. Burr holes were drilled in the skull on both sides over the lateral ventricles by using the following coordinates: 0.8 mm posterior to bregma, 1.5 mm lateral to saggital suture, 3.6 mm ventral from the surface of the brain [13]. STZ was dissolved in ACSF. STZ (3 mg/kg ICV) was injected bilaterally in two divided doses, on the first and the third day making the dose of 1.5 mg/kg each day. The concentration of STZ in ACSF was adjusted so as to deliver 10 µl of the solution. Rats in the control group were given ICV injection of same volume of ACSF on the first and third day as in STZ treated. After ICV injection, povidone-iodine solution was applied and the skin was sutured after second injection followed by daily application of Neosporin.

Drug Administration

The animals were divided into 7 different groups (n= 6 to 8) in each group viz Group1: Normal control animals treated with normal saline; Group 2: Control animals received an equivalent volume of vehicle for streptozotocin i.e. artificial CSF (ACSF) on day 1 and day 3; Group 3: animals received intra cerebro ventricular injection of streptozotocin (ICV-STZ) 3mg/kg on day 1 and day 3 as a positive control; Group 4, 5 and 6: ICV-STZ treated rats being administered Ethanol extract of *Holarrhena antidysenterica* seeds (EHHA)(100, 200 and 300 mg/kg; p.o. respectivily) to rats for 28 days daily; Group7: ICV-STZ treated rats received Donepezil (3 mg/kg/day p.o.) for 9 days [12].The doses of EEHA were selected on the basis of literature reports [14, 15]. EEHA was prepared as aqueous suspension with 1% w/v Tween 80. EEHA treatment was started on day 1, after the STZ-ICV injection and continued till end of the study i.e. 28 days. Memory impairment was assessed by elevated plus maze on days 27th and 28th day. Novel object recognition test and Locomotor activity was assessed on 28th day. After behavioral experiments; animals were sacrificed by cervical dislocation and decapitated. Brain was isolated with the help of micro-spatula and immediately washed with ice cold normal saline. Brain homogenate was prepared for estimating the biochemical parameters.

Behavioral Assessment
Elevated Plus Maze

Memory acquisition and retention was tested using elevated plus maze test on 27th and 28th day. The elevated plus maze consisted of two open arms (50 cm × 10 cm) and two enclosed arms (50 cm × 10 cm × 40 cm) extending from a central platform (10cm²). The maze was elevated to a height of 50 cm from the floor.

On the first day, each rat was placed at the end of either of the open arms, facing away from the central platform. Transfer latency (TL) was defined as the time (in seconds) taken by the animal to move from the open arm into one of the covered arms with all of its four paws. The cut off time to reach the closed arm is 90 sec. In case the rat does not locate the closed arm in 90 sec, it is gently guided to one of the closed arm. The rat was allowed to explore the maze for 20 s and then return to home cage. Retention of this learned task (memory) was examined 24 hours after the learning trial. Significant reduction in Transfer latency (TL) value indicates improvement of memory [16, 17].

Novel Object Recognition Task

Object Recognition (OR) may be performed in any simple box, with or without a transparent wall (if it is the case, the animal is to be observed from above). A typical apparatus has a 50cm high, 40 x 60cm box made of wood (or plastic) with a frontal glass wall, the inside of which is painted with clear colors. Usually the recognition objects are made of plastic or metal to allow easy cleaning between sessions with different animals [18]. Object exploration was defined as the rats sniffing, licking or touching the objects with forepaws whilst sniffing but not by leaning against, turning around, standing or sitting on the objects [19]. The exploration time (s) of each object in each trial was recorded manually using two stopwatches and the following factors were calculated: $E1$ = the total exploration time of both objects in the acquisition trial ($EA1 + EA2$), $E2$ = the total exploration time of both objects in the retention trial ($EA + EB$), and H= the habituation of exploratory activity ($E1 - E2$). The index of habituation to the familiar object measures differences between the average time spent in exploring the objects in the acquisition and the retention trials. DI = discrimination index ($EB - EA$)/($EB + EA$), the discrimination index represents the difference in exploration time expressed as a proportion of the total time spent exploring the two objects in the retention trial [20].

Open Field Activity

The apparatus consisted of a square arena [56 × 56 × 20 [H] cm] made of black wood and its floor was divided by lines into 16 squares that allowed the definition of central and peripheral parts. At the beginning of the session, each rat was placed in the centre of the arena and its activity was recorded for 5 min and the ambulation score was measured. Ambulation, measured as the numbers of marked floor segments that each animal entered with at least its front two paws during each 5 min period [21].

Biochemical estimations
Brain homogenate preparation

All animals were sacrificed at the end of study i.e. 4th week (28th day) and whole brain was immediately dissected out, rinsed with ice cold saline (0.9% sodium chloride)to remove the blood. The brain

was weighed and 10% tissue homogenate was prepared with chilled phosphate buffer, pH 7.4. The homogenates were centrifuged at 10,000×g at 4°c in cooling centrifuge for 20 minutes and the clear supernatant of homogenates was used to measure AChE activity [22], Thiobarbituric acid reactive substances (TBARS) [23], Glutathione (GSH) [24]and Total protein determination [25].

Estimation of AChE activity in brain

Cholinergic dysfunction was assessed by acetyl cholinesterase activity. The quantitative measurement of acetylcholinesterase levels in the whole brain homogenate was estimated according to the method of Ellman [22].

Brain thiobarbituric acid reactive substances (TBARS)

The brain was homogenized and centrifuged at 10,000×g at 4°c in cooling centrifuge for 20 minutes. The clear supernatant was used for the measurement of thiobarbituric acid reactive substances (TBARS) at absorbance 532 nm by using U.V/Visible spectrophotometer (Shimadzu 1700, Singapore). The concentrations were determined using a standard curve of 1, 1, 3, 3-tetramethoxypropane and the results were expressed as µM/ml [23].

Reduced glutathione (GSH) in brain

The GSH assay was performed by the method Ellaman et al. Supernatant was used for the measurement of GSH at absorbance 412 nm by using U.V/Visible spectrophotometer. The concentrations were determined using a standard curve of reduced glutathione and the results were expressed as µM/ml [24].

Protein determination

Protein concentration was estimated according to the method of Lowry et al. using BSA (bovine serum albumin) as a standard [25].

Statistical Analysis

All the results are expressed as Mean ± SEM. The data of all the groups were analyzed by one way ANOVA followed by Tukey's test using software Graph Pad prism 6 (Graph Pad Prism 6 Software). A value of $P<0.05$ was considered to be significant.

RESULTS
Effect of EEHA on Mean Transfer Latency using EPM

Transfer latency (TL) on day 2 (33.875 ± 0.27) was significantly ($p<0.05$) decreased in the normal saline treated control group when compared with that on day 1 (39.231 ± 0.59) acquisition trial. There was no significant difference in the transfer latency of the normal group and ACSF control group. STZ-ICV administration to rats caused the impairment of memory to rat as shown by higher TL value as compared to ACSF group and normal control group. EEHA administration (100 mg/kg, p.o.) did not show any statistically significant changes in TL as compared to STZ group rats. However, administration of EEHA (200 and 300 mg/kg, p.o.) for 28 successive days to separate groups of STZ treated rats caused reduction in TL ($p<0.05$) as compared to STZ group. Donepezil treatment also improved memory performance of rat in Elevated Plus Maze (Figure 1).
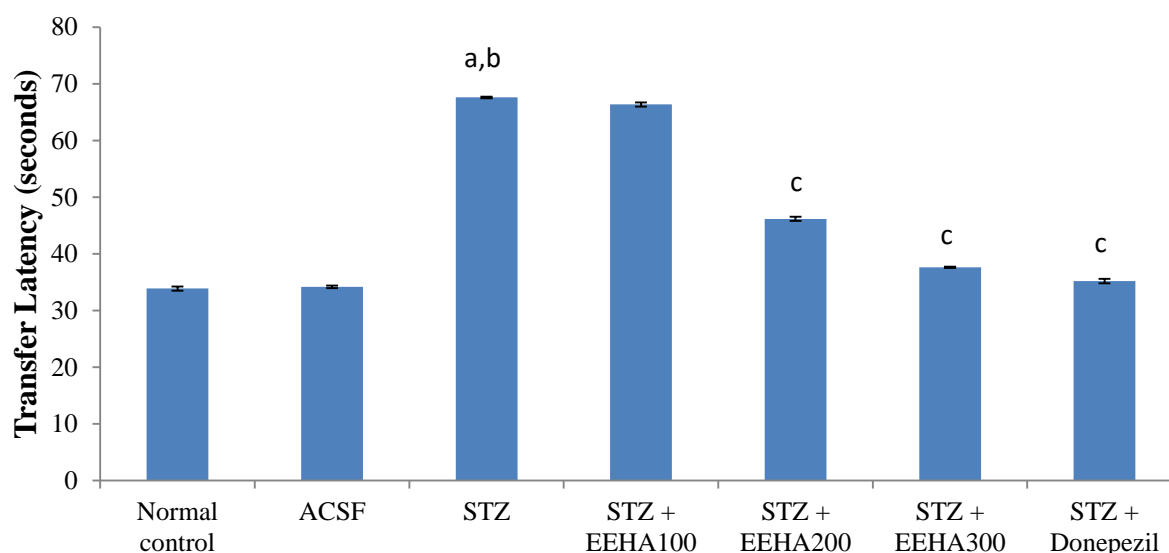

Fig 1: Effect of EEHA on Mean Transfer Latency using Elevated plus Maze
Values are expressed as mean ± SEM (n=6), [a] indicates p<0.05 in comparison to control; [b] indicates p<0.05 in comparison to ACSF; [c] indicates p<0.05 in comparison to STZ, (One way ANOVA followed by Tukey's test). EEHA indicates ethanol extract of *Holarrhena antidysenterica* ; ACSF indicates artificial cerebrospinal fluid, STZ streptozotocin.

Effect of EEHA on Exploration time and Discrimination Index using Novel Object Recognition Task

Acquisition trial:

All groups of rat spent equivalent time exploring the identical objects (left and right) in the acquisition phase. Statistical analysis showed no significant difference in time spent exploring the identical objects in the acquisition phase in any group (Figure 2)

Retention trial:

Normal and ACSF treated rats spent significantly (*p*<0.05) longer exploring the novel object compared with the familiar object. The ability to discriminate familiar and novel objects was abolished following STZ treatment, whereby there was no significant difference in exploration of the novel and familiar object. Administration of EEHA at dose (100 mg/kg; p.o.) failed to improve the STZ-induced impairment. However administration of EEHA (200 and 300 mg/kg; p.o) significantly attenuated the STZ-induced impairment such that a significant increase in time spent exploring the novel object compared with the familiar object was observed (*p* <0.05). Donepezil also improved memory performance in retention trial (Figure 3).

Discrimination Index:

A one-way ANOVA revealed a significant effect of treatment on DI. The DI was significantly (p<0.05) reduced following STZ-ICV induced Alzheimer's rats and EEHA (100 mg/kg; p.o.) rats. EEHA (200 and 300 mg/kg; p.o.) treated rats elevated DI as compared to STZ treated rats. Donepezil also showed rise in DI (Figure 4).
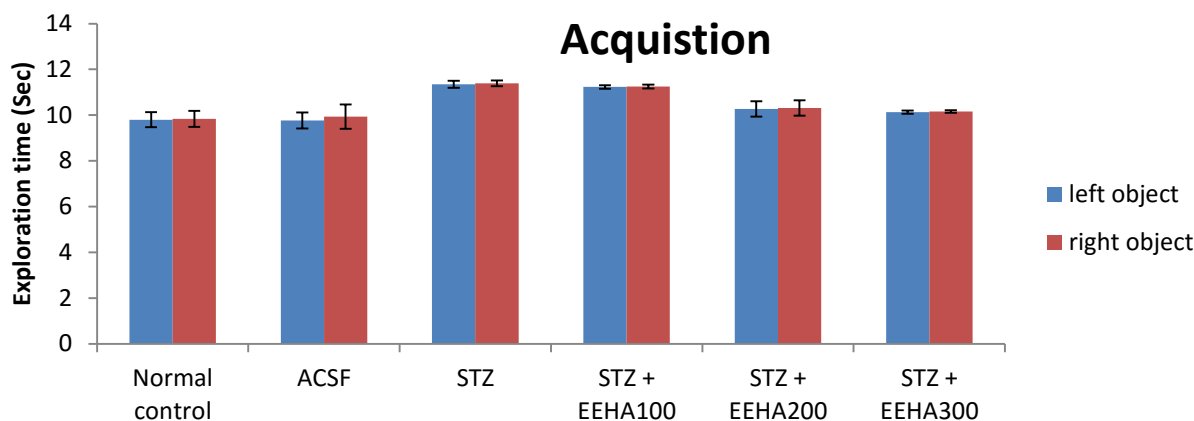

Fig 2: Effect of EEHA on Exploration time in Acquisition trial using Novel Object Recognition Task
Values are expressed as mean ± SEM (n=6),(One way ANOVA followed by Tukey's test). EEHA indicates ethanol extract of *Holarrhena antidyseneterica*; ACSF indicates artificial cerebrospinal fluid, STZ streptozotocin.
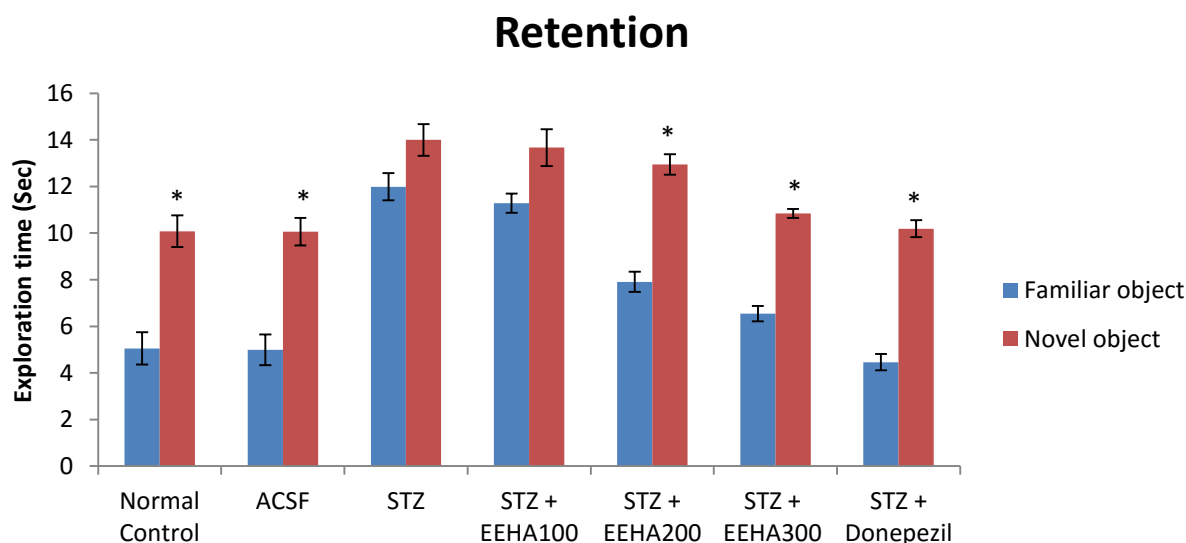

Fig 3: Effect of EEHA on Exploration time in Retention trial using Novel Object Recognition Task
Values are expressed as mean ± SEM (n=6), *p<0.05 significant difference in time spent exploring the novel compared with the familiar object, (One way ANOVA followed by Tukey's test). EEHA indicates ethanol extract of *Holarrhena antidysenetrica*; ACSF indicates artificial cerebrospinal fluid, STZ streptozotocin.
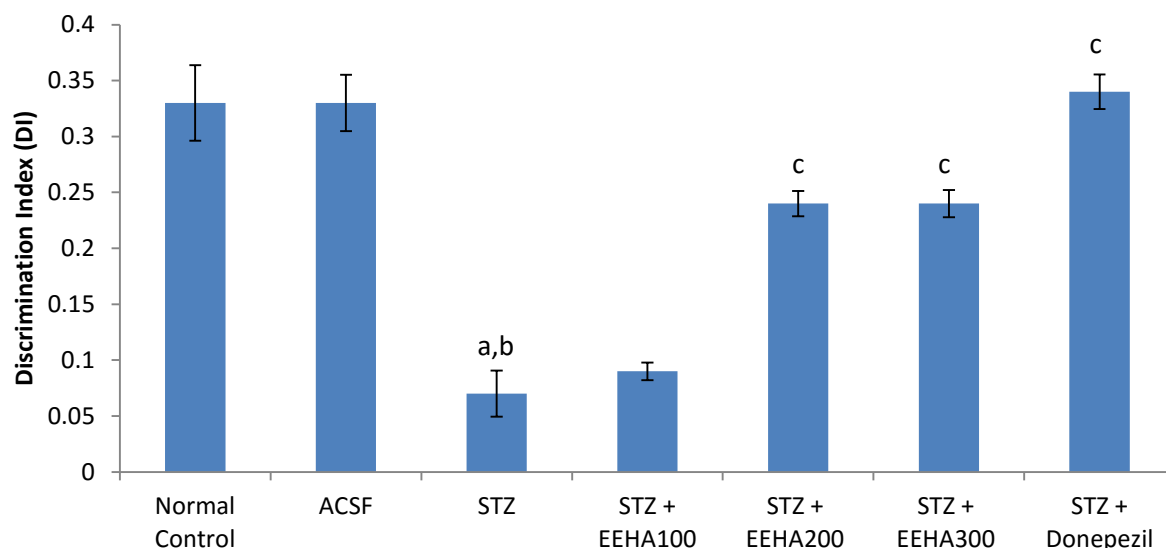
Fig 4: Effect of EEHA on Discrimination Index using Novel Object Recognition Task
Values are expressed as mean ± SEM (n=6), [a] indicates $p<0.05$ in comparison to control; [b] indicates $p<0.05$ in comparison to ACSF; [c] indicates $p<0.05$ in comparison to STZ, (One way ANOVA followed by Tukey's test). EEHA indicates ethanol extract of *Holarrhena antidysenterica* ; ACSF indicates artificial cerebrospinal fluid, STZ streptozotocin.

Effect of EEHA on Ambulation in Open Field test

Open field test usually measure general behaviors and ambulatory behaviors of rodents. All the groups showed no statistical significant difference in ambulatory score (Figure 5).
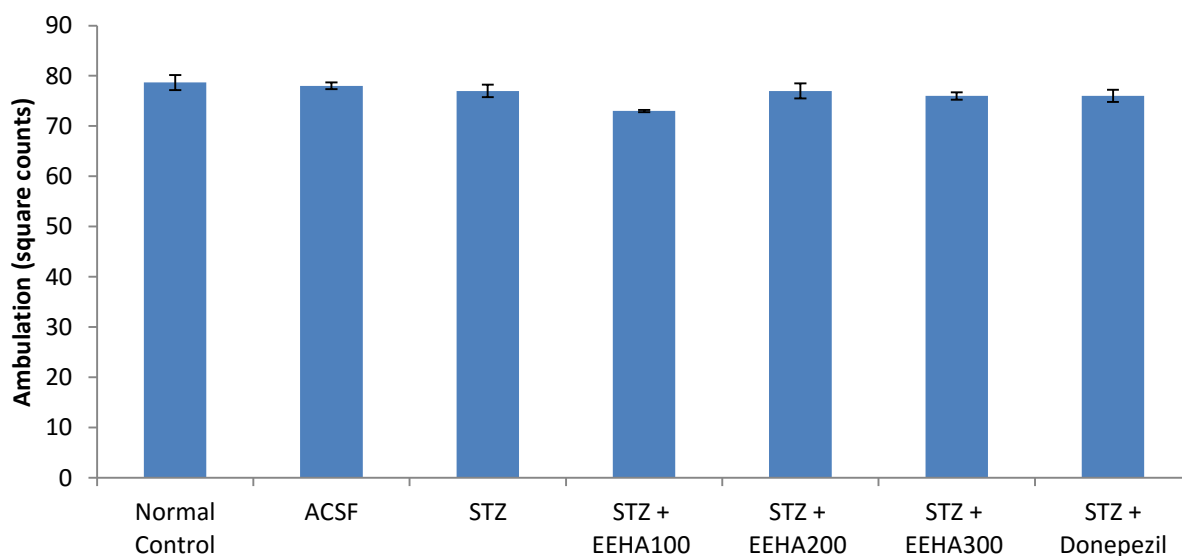

Fig 5: Effect of EEHA on Ambulation in Open field test
Values are expressed as mean ± SEM (n=6), (One way ANOVA followed by Tukey's test). EEHA indicates ethanol extract of *Holarrhena antidysentrica*, ACSF indicates artificial cerebrospinal fluid, STZ streptozotocin.

Effect of EEHA on brain AChE level

The normal and ACSF group showed no statistical difference in AChE level when compared with each other. STZ administration caused a marked increase in the AChE level as compared to normal and ACSF group. Administration of EEHA (100, 200 and 300 mg/kg, p.o.) for 28 days to the separate groups of STZ ($p<0.05$) significantly decrease the level of AChE as compared to the diseased group. Donepezil treatment for 9 days also improved brain AChE level of rats (Table 1).

Effect of EEHA on brain TBARS level

The levels of MDA in brain of normal group and ACSF group showed no statistical difference. STZ-ICV administration caused ($p<0.05$) significant increase

in brain MDA levels as compared to ACSF group and normal control group. However, administration of EEHA (100, 200 and 300 mg/kg, p.o.) for 28 successive days to separate group of STZ treated rats prevented the rise in MDA levels in a dose dependent manners (p<0.05) as compared to STZ group. Donepezil treatment also caused prevention of rise in brain TBARS level of rats (Table 1).

Effect of EEHA on brain GSH level

ICV administration of ACSF had no significant effect on brain GSH levels as compared to normal group. STZ administration caused marked depletion of GSH level as compared to ACSF and normal group of rats (p<0.05). However, administration of EEHA (100, 200 and 300 mg/kg, p.o.) for 28 successive days to separate groups of STZ (p<0.05) prevented the GSH depletion when compared to STZ group. Donepezil treatment for 9 days also caused rise in the brain GSH level of rats (Table 1).

Table 1: Effect of EEHA treatment on the biochemical indices in intracerebroventricular streptozotocin treated rats

Treatment	AChE Activity (µM/min/mg of protein)	TBARS (µM/ml)	GSH (µM/ml)
Normal Control	2.64 ± 0.02	10.85 ± 0.14	159.39 ± 3.57
ACSF	2.65 ± 0.01	11.09 ± 0.18	156.08 ± 2.16
STZ	$3.74 \pm 0.02^{a,b}$	$28.34 \pm 0.43^{a,b}$	$97.42 \pm 3.45^{a,b}$
STZ + EEHA100	3.29 ± 0.02^{c}	23.94 ± 0.17^{c}	118.33 ± 1.58^{c}
STZ + EEHA200	3.06 ± 0.01^{c}	19.82 ± 0.18^{c}	132.96 ± 2.16^{c}
STZ +EEHA300	2.76 ± 0.02^{c}	15.29 ± 0.41^{c}	147.31 ± 1.28^{c}
STZ + Donepezil	2.71 ± 0.07^{c}	11.65 ± 0.15^{c}	152.2 ± 0.79^{c}

Values are expressed as mean ± SEM (n=6) (One way ANOVA followed by Tukey's test). EEHA indicates ethanol extract of *Holarrhena antidysentrica*, ACSF indicates artificial cerebrospinal fluid, STZ streptozotocin.[a] indicates p<0.05 in comparison to control; [b] indicates p<0.05 in comparison to ACSF; [c] indicates p<0.05 in comparison to STZ

DISCUSSION

Holarrhena antidysenterica Linn is commonly known as Tellicherry Seeds (English) and Kurchi (Hindi) and belongs to family Apocynaceae. The seeds of *Holarrhena antidysnterica* Linn contain conessine ($C_{24}H_{40}N_2$), iso conessine ($C_{24}H_{40}N_2$), conessidine/isoconessimine ($C_{23}H_{38}N_2$), conarrhimine ($C_{21}H_{34}N_2$) [11]. The seeds are used as an astringent, anthelmintic, antidontalgic, stomachic, febrifuge, antidropsical, diuretic, dyspepsia, chest infections and as a remedy in diseases of the skin and spleen. It is a well known drug for amoebic dysentery and other gastric disorders. It is also indicated in diarrhea, indigestion, flatulence and colic [26]. Hence, considering anticholinsterase and antioxidant activity of *Holarrhena antidysenterica* Linn, the present investigation was undertaken to evaluate efficacy of ethanol extract of seeds of *Holarrhena antidysenterica* Linn (EEHA) on dementia in experimental animals.

In the present study, STZ-ICV administration has shown deficits in learning and memory as indicated by Novel object recognition task and Elevated plus Maze study. Streptozotocin is a glucose-like molecule with a nitrosourea moiety. It has been reported that ICV injection of STZ causes chronic reductions (10–30%) in glucose and glycogen metabolism in cerebral cortex and hippocampus [27]. These effects are associated with significantly reduced brain oxidative metabolism and progressive deficit in learning and memory and cerebral energy balance. Grünblatt *et al.;* has reported that ICV administration of STZ produces severe abnormalities in metabolic pathways being under control of the IR

signaling cascade in the rat brain [28]. STZ-ICV treated rat has been described as an appropriate model for sporadic Alzheimer's disease (SAD) which is characterized by progressive deterioration of memory, cerebral glucose and energy metabolism [29]. There was no significant difference found in the locomotor activity of control, ACSF, STZ and EEHA treated rats, which rules out the possibility of interference by locomotor activity changes in elevated plus maze and novel object recognition task.

The AD is characterized by alterations at the level of various neurotransmitters. The most severely affected is the cholinergic system, which is responsible for the storage and retrieval of items in memory and its degradation correlates well with the severity of cognitive and memory impairment [30,31]. AChE activity, an enzyme responsible for degradation of ACh, which is in tune with earlier reports [32, 33]. The enhancement of cholinergic activity by the inhibition of AChE enzyme is the mainstay of symptomatic treatment of dementia [34]. In the present study, the decrease was found in AChE activity in brain during EEHA administration which leads to increase in cholinergic activity to facilitate learning supports the finding of Das *et al.;* 2005 [35]. STZ treatment showed significant increase in AChE activity in whole brain which is consistent with earlier studies [12, 36]. AChE activity was found to be restored by administration of three doses of EEHA that that may be linked to its ability to augment in cholinergic activity. Oxidative damage to the rat synapse in different regions of brain has been reported to contribute to cognitive deficits

[37]. Dementic rats showed increase in TBARS, AChE and decrease in GSH levels. EEHA treated rats showed the decreased TBARS and AChE levels and increased GSH level. Administration of three doses of EEHA reversed the STZ induced oxidative stress which can be linked to free radical scavenging activity of EEHA.

CONCLUSION

The results of the study elucidate that *Holarrhena antidysenterica* Linn avert the STZ induced dementia in rats by virtue of pro-cholinergic and antioxidant activities.

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