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Gugulipid, an extract of *Commiphora whighitii* with lipid-lowering properties, has protective effects against streptozotocin-induced memory deficits in mice☆

Gunjan Saxena [a], Sheelendra Pratap Singh [a], Raghvendra Pal [b], Stayawan Singh [a], Ram Pratap [c], Chandishwar Nath [a,*]

[a] *Division of Pharmacology, Central Drug Research Institute (CDRI), Lucknow 226001, India*
[b] *Division of Pharmaceutics, Central Drug Research Institute (CDRI), Lucknow 226001, India*
[c] *Division of Medicinal Chemistry, Central Drug Research Institute (CDRI), Lucknow 226001, India*

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Abstract

Gugulipid, an ethyl acetate extract of the resin of plant *Commiphora whighitii* is an established hypolipidemic agent in clinical practice. The major constituent of gugulipid is guggulsterone [4, 17 (20)-pregnadiene-3, 16-dione]. It has been observed recently that patients receiving lipid-lowering drugs like statins have a reduced risk of dementia. Therefore, the present study was planned to explore the potential of gugulipid as cognitive enhancer. Gugulipid (12.5, 25 and 50 mg/kg, p.o.) showed dose dependent improvement in scopolamine-induced deficits in passive avoidance test. The maximal effective dose of gugulipid i.e. 50 mg/kg, p.o. was used for further studies on streptozotocin (STZ) model of dementia in mice. Gugulipid was investigated for its effect on learning and memory, parameters of oxidative stress (GSH and MDA) and acetylcholinesterase (AChE) activity in the STZ (ic)-treated mice. Intracerebral (ic) injections of STZ (0.5 mg/kg) on 1st and 3rd day caused significant deficit in memory in passive avoidance and Morris water maze test after the 14th day of first dose. In passive avoidance, transfer latency time (TLT) was not increased on retention trials in STZ (ic) group while gugulipid treatment resulted in significant increase in TLT on retention trials in STZ (ic)-treated mice. In Morris water maze test the latency time to reach platform in STZ (ic)-treated mice was significantly higher than control and vehicle (artificial CSF). Pre-treatment of gugulipid (50 mg/kg, p.o.) daily for 14 days started with the first dose of STZ (ic), significantly prevented STZ (ic)-induced memory deficit. Post-treatment i.e. after 14 days of first dose of STZ (ic) of gugulipid (50 mg/kg, p.o.) significantly decreased the latency time indicating anti-dementia activity. Effect of gugulipid and STZ in visible platform test was similar to those seen with hidden platform. Gugulipid and STZ-treated mice did not cause significant change in locomotor activity. Furthermore, STZ (ic) resulted into increase in AChE activity, low level of GSH and high concentration of MDA in brain on 21st day as compared to control. Gugulipid treatment caused significant decrease in AChE activity, low level of MDA and high concentration of GSH in brain following STZ (ic) as compared to vehicle administration in STZ (ic)-treated mice. The study demonstrated that gugulipid has significant protective affect against streptozotocin-induced memory deficits model of dementia that can be attributed to anti-oxidant and anti-AChE activity of gugulipid. These observations suggest gugulipid as a potential anti-dementia drug (CDRI, Lucknow has obtained US patent No. 6896901 for use of gugulipid as cognitive enhancer).

Keywords: Gugulipid; Memory; Streptozotocin; Anti-oxidant; Anti-cholinesterase

1. Introduction

Commiphora whighitii (Family: *Burseraceae*) commonly called, as gum guggulu is highly valued in Ayurveda an Indian system of medicine. The gum resin exudate of *C. whighitii* tree has been used in Ayurvedic medicine for more than 2000 years to treat a variety of ailments like obesity, lipid disorders, rheumatoid arthritis (Dev, 1987). Experimental studies with extracts and fractions of guggulu demonstrated anti-inflammatory activity (Gujral et al., 1960).

Gugulipid, an ethyl acetate extract of the resin of plant *C. whighitii* is an established hypolipidemic agent. The major

☆ CDRI Communication No. 6978.
* Corresponding author. Tel.: +91 522 2212411 18x4434.
E-mail address: cnathcdri@rediffmail.com (C. Nath).

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constituent of gugulipid is guggulsterone [4, 17 (20)-pregnadiene-3, 16-dione]. The mechanisms implicated for lipid-lowering effect of gugulipid are stimulation of hepatic lipases and receptor mediated catabolism of low-density lipoproteins, and suppression of hepatic cholesterol biosynthesis (Nityanand and Kapoor, 1973). The plethora of pharmacological effects of guggulu particularly anti-dyslipidemic effect is suggestive of its potential as cognitive enhancer. Patients receiving lipid-lowering drugs like statins have a reduced risk of dementia (Jick et al., 2000). Lipids account for half of the dry matter of the brain and are integral to the myelin sheath and synapses. Anything that affects the balance of cerebral lipid metabolism could have profound effects on brain function. High cholesterol is also associated with elevated beta-amyloid, the hallmark of AD (Borek, 2006). Experimental studies have shown that cholesterol-fed wild-type rabbits develop brain pathology similar to Alzheimer's disease, which is supported by human studies, showing that statin therapy reduces the risk of Alzheimer's disease (Parale et al., 2006; Raja et al., 2004; Masse et al., 2005). However, study on gugulipid for its effects on memory functions and potential for dementia disorders is lacking. Therefore, the present study was planned to investigate effect of gugulipid on intracerebral-streptozotocin (STZ-ic) model of dementia in mice to explore the potential of gugulipid as cognitive enhancer.

Intracerebroventricularly (icv) administered streptozotocin (STZ)-induced deficit in learning and memory functions is a commonly used experimental model of dementia in rodents. It is reported that icv injection of STZ in a sub-diabetogenic dose in rats causes prolonged impairment of brain glucose and energy metabolism (Geert et al., 1998). This is accompanied by impairment in learning and memory in addition to decreased choline acetyltransferase levels in the hippocampus (Blockland and Jolles, 1993; Lannert and Hoyer, 1998). Some other studies are also associated with STZ (icv)-induced cognitive impairment with free radical generation in the brain of rats (Sharma and Gupta, 2001a,b). The effect of gugulipid on learning and memory function on passive avoidance and Morris water maze was studied in the STZ (ic)-treated mice along with the parameters of oxidative stress glutathione (GSH) and malondialdehyde (MDA) and acetylcholinesterase (AChE) enzyme activity in brain.

2. Methods

2.1. Animals

The experiments were carried out with adult male Swiss Albino mice weighing 25–30 g. The animals were kept in polyacrylic cage with 6 mice per cage and maintained under standard housing condition (room temperature 24–27 °C and humidity 60–65%) with 12 h light and dark cycle. There were 6 animals in each group. The food in the form of dry pellets and water were available ad libitum but food was not allowed during the experiment.

The animals were procured from the Laboratory Animal Services Division of Central Drug Research Institute, Lucknow. Experiments were performed according to the internationally followed ethical standards and approved by the research ethics committee of Central Drug Research Institute and CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

2.2. Tests employed for learning and memory functions

2.2.1. Passive avoidance test

The mice were subjected to a single trial passive avoidance test as described by Das et al. (2000). Briefly an animal was placed in the lighted compartment of a computerized shuttle box (Columbus Instruments, Ohio, USA) provided with a software programme PACS 30. An automated guillotine door isolates the compartment lighted at intensity of 8 (scale of 0 — off and 10 — brightest provided in the PACS 30 software) from the dark compartment. After an acclimatization period of 30 s the guillotine door automatically opened and after entry into the dark compartment automatically shut the door and the subject received a low intensity foot shock (0.5 mA; 10 s). Infrared sensors monitored the transfer of animal from one compartment to another, which was recorded as transfer latency time (TLT) in seconds. The 1st trial was for acquisition and retention was tested by a 2nd trial given 24 h after the 1st trial. More trials after 2nd trial at 24 h interval were given to test retention in STZ-treated mice. The shock was not delivered in the retention trials to avoid reacquisition. The criterion for learning was taken as an increase in the TLT on retention (2nd or subsequent) trials as compared to acquisition (1st) trial.

2.2.2. Morris water maze

The Morris water maze consisted of a large circular black pool 120 cm diameter, 50 cm height, filled to a depth of 30 cm with water at 26±2 °C temperature, which was placed in a darkened room. Within the pool, a submerged black colored round platform of 8 cm diameter was placed 1 cm below surface of water. The water was colored by a non-toxic black dye to hide the platform location. The mice could climb on the platform to escape from the necessity of swimming. Trial was given for 5 consecutive days in order to train mice in Morris water maze. Same starting position was used on each trial. The mice were given a maximum time of 60 s (cut off time) to find the hidden platform (Chen et al., 2002) and were allowed to stay on it for 30 s. The experimenter put the mice that failed to locate the platform onto it. The position of the mice in the pool was automatically registered on a video tracking system (Videomax, Columbus Inc. USA). The animals were given a daily session of 3 trials per day. Latency time to reach the platform was recorded in each trial. Latency time of the last trial of each session is shown in the Results section. Significant decrease in latency time from that of 1st session was considered as successful learning. One set of experiment was conducted in similar manner with visible platform (Hauben et al., 1999).

2.3. Induction of memory deficits (dementia)

2.3.1. Scopolamine-induced memory deficits

Scopolamine (3 mg/kg, i.p.) was administered 5 min prior to the 1st trial (acquisition) to produce amnesia in passive avoidance test (Das et al., 2005).

2.3.2. Intracerebral (ic) administration of streptozotocin (STZ)

The mice were anesthetized with chloral hydrate (300 mg/kg, i.p.). STZ was injected (0.5 mg/kg) intracerebrally (ic) according to the method of Haley and McCormick (1957). The same dose of STZ was repeated after 48 h of first dose. STZ was dissolved in an artificial CSF. This solution was made freshly just before the ic administration. The volume of ic administration was 10 μl. After 14 days of first dose, the animals were subjected to testing of learning and memory functions in passive avoidance or Morris water maze test.

2.4. Spontaneous locomotor activity

Each animal was observed for 10 min after a period of 30 min for acclimatization in Optovarimex activity meter (Columbus Inc USA).

2.5. Dose schedule of gugulipid

Gugulipid was prepared in Central Drug Research Institute (CDRI) Lucknow, India (CDRI, Lucknow has obtained US patent no. 6896901 for the use of gugulipid as cognitive enhancer). Gugulipid was administered orally as aqueous suspension with 1% methylcellulose in the following dose schedule for different experimental setup.

2.5.1. In scopolamine model

Gugulipid was administered orally in doses 12.5, 25 and 50 mg/kg 1 h prior to the 2nd trial in passive avoidance test to observe its anti-amnesic effect.

2.5.2. In STZ (ic) model

In passive avoidance test, mice were subjected to acquisition trial (1st trial) on the 14th day from the first ic injection of STZ. To observe effect on STZ-induced dementic mice, gugulipid was administered orally daily at a dose of 50 mg/kg 1 h prior to the 2nd, 3rd, 4th and 5th retention trials that were given on the 15th, 17th, 19th and 21st days starting from the first ic injection of streptozotocin. In a separate set of experiment, preventive potential of gugulipid was evaluated by its administration at a dose of 50 mg/kg, p.o. daily for 14 days starting from the first day of STZ (ic) injection.

In Morris water maze test, gugulipid was administered orally at a dose of 50 mg/kg 1 h prior to daily session of trials that were given on the 15th, 16th, 17th, 18th and 19th days starting from the first ic injection of streptozotocin. In another set, gugulipid was administered orally at a dose of 50 mg/kg daily from the day of the first ic injection of streptozotocin to study the preventive effect of gugulipid till the 14th day. Similar protocol of gugulipid administration was also followed with visible platform test.

In one set of experiments, Gugulipid was administered daily in a dose of 50 mg/kg, p.o. for 4 days in naïve mice to study its effect on memory functions in Morris water maze test.

Control (no treatment), vehicle (10 ml/kg, p.o. of 1% methyl cellulose aqueous suspension) and artificial CSF (10 μl ic on 1st and 3rd day) groups were included in the study according to the experimental design.

Thus, the groups in the study with STZ model were control (no treatment), artificial CSF (ic), STZ (ic), 1% methyl cellulose in STZ (ic) treated (STZ (ic)+vehicle) as vehicle control, STZ (ic) in gugulipid pre-treated (gugulipid+STZ) for preventive effect of gugulipid and gugulipid in STZ (ic) pre-treated (STZ+gugulipid) for post-treatment curative effect.

2.6. Estimation of biochemical parameters

Acetylcholinesterase (AChE) and biochemical parameters of oxidative stress malondialdehyde (MDA) and glutathione (GSH) were estimated in the brain on the 21st day after the STZ injection.

2.6.1. Brain tissue preparation

The mice were decapitated under ether anesthesia. The brain was exposed from its dorsal side by incising the skull. The whole brain was quickly removed from each mouse and cleaned with chilled saline over the ice. A 10% (w/v) homogenate of brain samples (0.03 M sodium phosphate buffer, pH-7) was prepared by using an Ultra-Turrax T25 (USA) homogenizer at a speed of 9500 rpm. 500 μl of the brain homogenate was mixed with 1% Triton X-100 (1% w/v in 0.03 M sodium phosphate buffer, pH-7). Both the TritonX-100-treated and non-TritonX-100-treated brain homogenate samples were centrifuged at 100,000 *g* at 4 °C in a Beckman Ultracentrifuge (LE 80, USA) using a fixed angle rotor (80 ti) for 60 min. Supernatant of TritonX-100-treated sample was collected and stored at 4 °C for AChE estimation. 1 ml of supernatant of non-TritonX-100-treated sample was mixed with equal amount of 10% TCA and stored at 4 °C overnight to estimate MDA and GSH. TCA-treated samples were then centrifuged (Remi cold centrifuge) at 2000 *g* for 30 min at 4 °C. The supernatant was used for MDA estimation. The pellets were dissolved in 0.1 N NaOH and were used for GSH estimation.

2.6.2. AChE assay

The assay of AChE in the supernatant of TritonX-100-treated samples was performed by Ellman's (1961) method. However, a minor change in the final concentration (1 mmol/l) of substrate, acetylthiocholine iodide and chromophore, 5′, 5′-dithiobis-(2-nitrobenzoic acid) (DTNB) (1 mM) led to a better kinetic profile. Henceforth, studies were performed with these modified concentrations (Das et al., 2002, 2005). A kinetic profile of the enzyme activity was measured spectrophotometrically (Shimadzu, USA) at 412 nm at an interval of 15 s. The assay for each sample was run in duplicate and each experiment was performed three times. One unit of AChE activity was defined as the number of micromoles (μmol) of acetylthiocholine iodide hydrolyzed per minute (min) per milligram (mg) of protein. The specific activity of AChE is expressed in μmol/min/mg of protein.

2.6.3. Measurement of MDA

MDA, which is a measure of lipid peroxidation, was measured spectrophotometrically by the method of Ohkawa et al. (1979) using 1, 1, 3, 3-tetraethoxypropane as the standard.

MDA was expressed as nmol per mg protein. The reagents acetic acid 1.5 ml (20%) pH 3.5, 1.5 ml thiobarbituric acid (0.8%) and 0.2 ml sodium dodecyl sulfate (8.1%) were added to 0.1 ml of processed tissue sample. The mixture was then heated at 100 °C for 60 min. The mixture was cooled under tap water and 5 ml of *n*-butanol: pyridine (15:1% v/v), 1 ml of distilled water was added. The mixture was shaken vigorously on vortex. After centrifugation at 4000 rpm for 10 min, the organic layer was withdrawn and absorbance was measured at 532 nm using spectrophotometer.

2.6.4. Measurement of glutathione

Glutathione was determined by its reaction with 5,5′-dithiobis (2-nitrobenzoic acid) (Ellman's reagent) to yield a yellow chromophore which was measured spectrophotometrically. Glutathione was measured spectrophotometrically (Ellman, 1959). To 0.1 ml of processed tissue sample, 2 ml of phosphate buffer (pH 8.4), 0.5 ml of 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) and 0.4 ml of double distilled water was added. The mixture was shaken vigorously on vortex and the absorbance read at 412 nm within15 min.

2.6.5. Protein estimation

Protein was measured in all the brain samples for GSH, MDA and AChE activity by the method of Lowry et al. (1951). Bovine serum albumin (BSA) (1 mg/ml) was used as standard and measured in the range of 0.01–0.1 mg/ml.

2.6.6. Blood glucose estimation

Blood was collected by tail prick and glucose was measured by Accu-Check Sensor comfort glucostrips (Roche Diagnostic, India) in control, CSF (ic), STZ (ic) and gugulipid STZ (ic)-treated groups, 14 days after the first dose of STZ (ic).

2.7. Statistical analysis

The results are expressed as mean±S.E.M. Statistical analysis of the Morris water maze, locomotor activity and biochemical values were performed by one-way analysis of variance (post test — Tukey). Data of passive avoidance test was analyzed by Student's test (paired).

3. Results

3.1. Effects of gugulipid pre-treatment on scopolamine-induced memory deficits in Passive avoidance test

The transfer latency time (TLT) was significantly increased on the 2nd trial as compared to the 1st trial in control group [$p<0.001$, $t=11.89$]. In scopolamine-treated group there was no significant increase in TLT on the 2nd trial (retention) as compared to the 1st trial (acquisition) [$p>0.05$, $t=0.47$]. Gugulipid at a dose of 50 mg/kg orally, in scopolamine pre-treated mice significantly increases the transfer latency time on the 2nd trial as compared to the 1st trial [$p<0.001$, $t=19.016$]. However, the effect of scopolamine was not significantly affected by lower doses 12.5 [$p>0.05$, $t=0.73$] and 25 [$p>0.05$, $t=2.18$] mg/kg, p.o. of gugulipid. There was no significant difference ($F(4,25)=0.43$, $p>0.05$)in TLT among the 1st trials of different groups (Fig. 1).

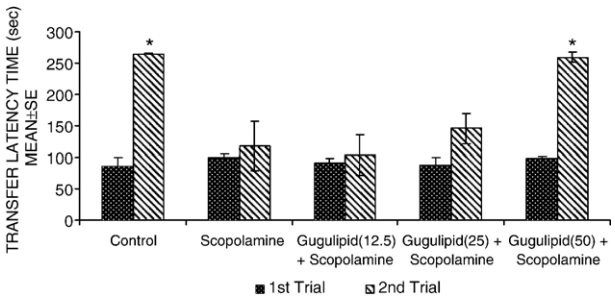

Fig. 1. Effect of different doses (12.5, 25 and 50 mg/kg, p.o.) of gugulipid (GUG) on scopolamine (3 mg/kg, i.p.)-induced deficit in memory in passive avoidance test. *Significant difference ($p<0.001$, two-tailed) from the 1st trial (Student's '*t*' test paired).

3.2. Effects of gugulipid on streptozotocin (ic)-induced memory deficits in Passive avoidance test

The transfer latency time was significantly increased on the 2nd and subsequent trials (retention) as compared to the 1st trial (acquisition) in control [$p<0.001$, $t=9.24–17.79$] and artificial CSF-treated group, [$p<0.001$, $t=8.98–16.366$]. In STZ-treated group, there was no significant increase on the 2nd to 5th retention trials as compared to the 1st trial [$p>0.05$, $t=0.77–1.87$] (Fig. 2a). Administration of vehicle (1% methyl cellulose) in STZ-treated mice did not show any significant change in TLT during retention trials [$p>0.05$, $t=0.1540–0.3165$]. Gugulipid (50 mg/kg, p.o.) administered daily for 14 days starting from the first day of STZ (ic) injection (Gugulipid+STZ), showed a significant increase in TLT on the 2nd–5th trials (retention) in comparison to that of the 1st trial (acquisition) [$p<0.01–0.001$, $t=3.44–4.91$]. In another set of experiments, gugulipid at a dose of 50 mg/kg orally, in STZ (ic) pre-treated mice (STZ+Gugulipid) significantly increased TLT on the 4th and 5th retention trials as compared to the 1st trial (acquisition) [$p<0.01$, $t=3.65$] (Fig. 2b).

3.3. Effects of gugulipid on learning and memory in Morris water maze test

Control (vehicle) and gugulipid (50 mg/kg, p.o. daily) reduced the latency time to reach platform. Significant decline in latency time occurred with treatment and days [two-way ANOVA, treatment $F(11)=3.13$, $p<0.01$; days $F(3)=10.56$, $p<0.001$]. However, significant decrease in latency time as compared to the 1st session occurred with gugulipid-treated group on the 2nd day while control group showed it on the 4th day [$F(2,15)=23.488$, $p<0.01$, Tukey test] (Fig. 3).

3.4. Effects of gugulipid on streptozotocin (ic)-treated mice in water maze test using visible platform

The latency time in the 3rd, 4th and 5th sessions were significantly lower than that of the 1st session in control [$F(4,25)=$

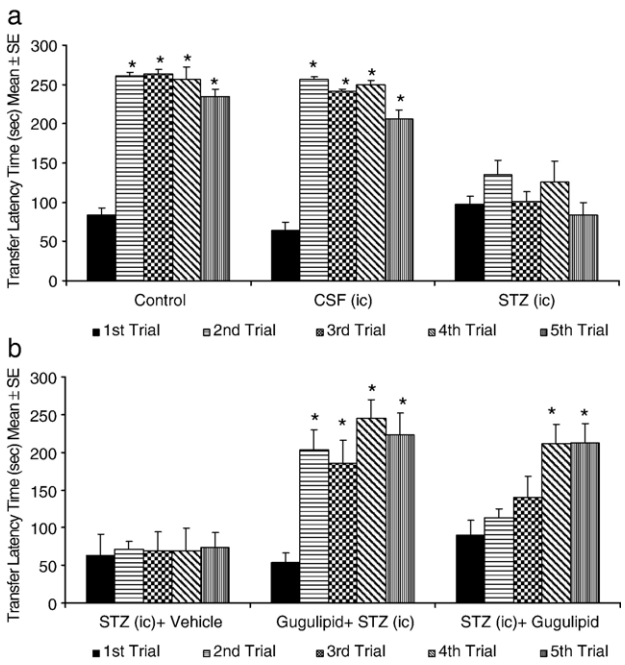

Fig. 2. (a) Transfer latency time (TLT) of control, artificial CSF (ic) and STZ (ic) in Passive Avoidance Task on the 1st trial (acquisition) and 2nd, 3rd, 4th, and 5th retention trials. *Significant difference ($p<0.001$, two-tailed) from the 1st trial of respective group (Student's '*t*' test paired). (b) Transfer latency time (TLT) of 1% methyl cellulose in STZ (ic)-treated (STZ (ic)+vehicle), STZ (ic) in gugulipid pre-treated (gugulipid+STZ) and gugulipid in STZ (ic) pre-treated (STZ+gugulipid) in Passive Avoidance Task on the 1st trial (acquisition) and 2nd, 3rd, 4th, and 5th retention trials. *Significant difference ($p<0.01$–0.001, two-tailed) from the 1st trial (Student's '*t*' test paired).

11.016, $p<0.01$, Tukey test], CSF [$F(4,25)=23.602$, $p<0.01$, Tukey test] and gugulipid (50 mg/kg, p.o. daily)-treated [$F(4,25)=7.303$, $p<0.01$, Tukey test] groups. There was no significant change in latency time to reach visible platform in STZ (ic)-treated mice in retention sessions (2nd to 5th) [$F(4,25)=0.3991$, $p>0.05$, Tukey test] in comparison to the 1st session. Administration of gugulipid in STZ (ic) pre-treated mice (STZ+Gugulipid) significantly decreased the latency time from the 3rd session as compared to the 1st session [$F(4,25)=4.725$, $p<0.01$, Tukey test] (Fig. 4).

3.5. Effects of gugulipid on streptozotocin (ic)-induced memory deficits in Morris water maze test

In control [$F(4,25)=2.430$, $p<0.001$, Tukey test] and CSF groups [$F(4,25)=23.588$, $p<0.001$, Tukey test] latency time in the 3rd, 4th and 5th sessions were significantly lower than that of the 1st session. There was no significant change in latency time to reach platform in STZ (ic)-treated mice in retention sessions (2nd to 5th) in comparison to the 1st session [$F(4,25)=1.538$, $p>0.05$, Tukey test] (Fig. 5a). Administration of vehicle (1% methyl cellulose) in STZ-treated mice did not show any significant change in latency time during retention sessions [$F(4,25)=1.726$, $p>0.05$, Tukey test]. Pre-treatment of gugulipid for continuous 14 days from the day of ic injection of STZ (Gugulipid+STZ) significantly decreased the latency time from the 1st session [$F(4,25)=5.426$, $p<0.01$, Tukey test]. Post-

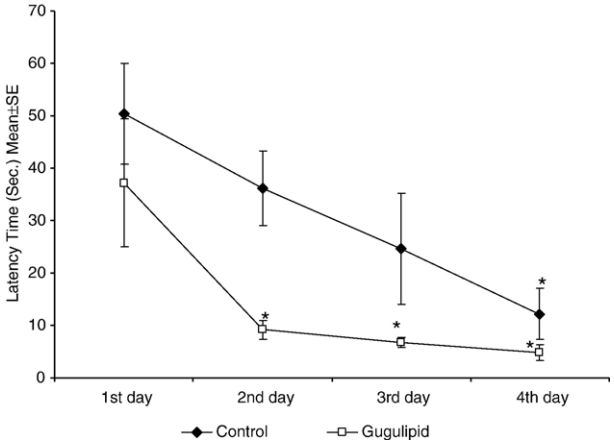

Fig. 3. Comparison of latency times of control and gugulipid-treated mice in Morris water maze test. *Significant difference ($p<0.01$) in latency time from the 1st session; ANOVA (two-way) followed by post-hoc analysis.

treatment of gugulipid in STZ (ic) pre-treated mice (STZ+Gugulipid) significantly reduced the latency time in retention sessions from the 1st session [$F(4,25)=38.164$, $p<0.001$, Tukey test] (Fig. 5b).

3.6. Locomotor activity

The spontaneous locomotor activity did not differ significantly [$F(2,15)=0.28$, $p>0.05$] between control, gugulipid (50 mg/kg, p.o.) and STZ-treated (14th day from the first dose) mice. The mean activity counts (±SE) of control, gugulipid and STZ-treated mice were 1030±8.95, 981±67.5 and 1010±43.66, respectively.

3.7. Estimation of parameters of oxidative stress in brain

3.7.1. MDA level

MDA level in the brain was estimated on day 21 after the first dose of STZ. The MDA levels for CSF control, STZ (ic) groups and vehicle-treated group were 23.09±1.62, 55.45±3.147 and 51.17±1.80 nmol/mg protein, respectively. The STZ (ic) mice and vehicle-treated STZ (ic) mice showed significant rise in levels of MDA as compared to control group. STZ (ic) groups-treated with gugulipid for 14 days (preventive) and

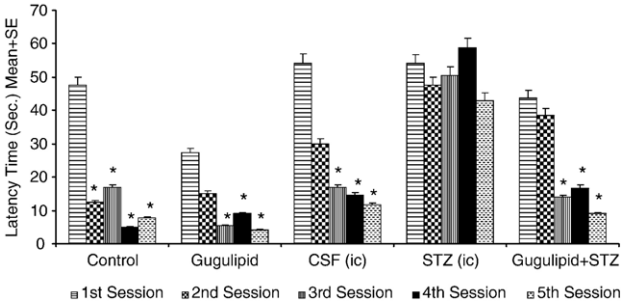

Fig. 4. Comparison of latency times of control, gugulipid, CSF, STZ (ic) and STZ+gugulipid-treated mice in Morris water maze test using visible platform. *Significant difference ($p<0.01$) in latency time from the 1st session; ANOVA (one-way) followed by Tukey test.

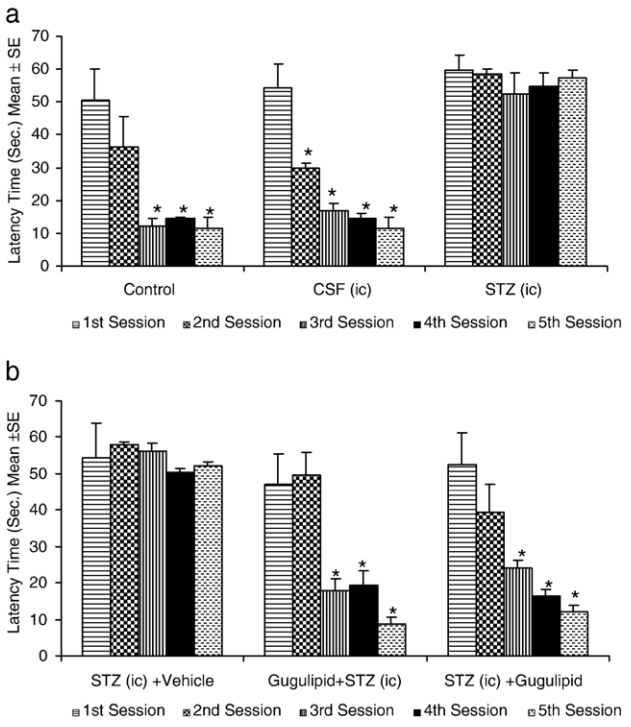

Fig. 5. (a) Comparison of latency times of control, artificial CSF (ic), and STZ (ic)-treated mice in Morris water maze test. *Significant difference ($p<0.001$) in latency time from the 1st session; ANOVA (one-way) followed by Tukey test. (b) Comparison of latency times of 1% methyl cellulose in STZ (ic)-treated (STZ (ic)+vehicle), STZ (ic) in gugulipid pre-treated (gugulipid+STZ) and gugulipid in STZ (ic) pre-treated (STZ+gugulipid) mice in Morris water maze test. *Significant difference ($p<0.01$–0.001) in latency time from the 1st session; ANOVA (one-way) followed by Tukey test.

gugulipid-treated STZ (ic) group (post-treatment) showed significant decrease in MDA level as compared to the STZ (ic) group [$F(5,30)=45.09$, $p<0.001$, Tukey test]. The MDA values of the gugulipid (50 mg/kg, orally) both pre-treated and post-treated STZ (ic) groups were 25.33±2.26 and 38.56±1.357 nmol/mg protein respectively (Fig. 6).

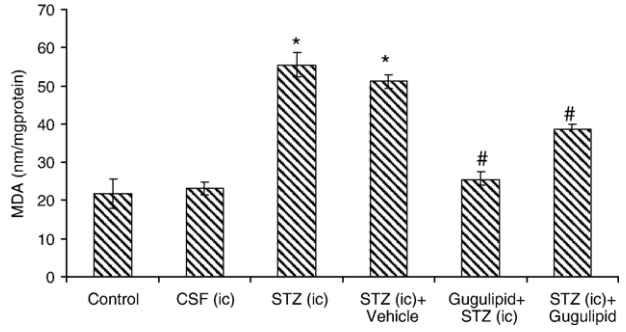

Fig. 6. MDA level in the brain of control, artificial CSF (ic), STZ (ic), 1% methylcellulose in STZ (ic)-treated (STZ (ic)+vehicle), STZ (ic) in gugulipid pre-treated (gugulipid+STZ) and gugulipid in STZ (ic)-treated (STZ+gugulipid) mice. *Significant difference ($p<0.05$) in MDA from control. #Significant difference ($p<0.001$) in MDA from STZ (ic) group; ANOVA (one-way) followed by Tukey test.

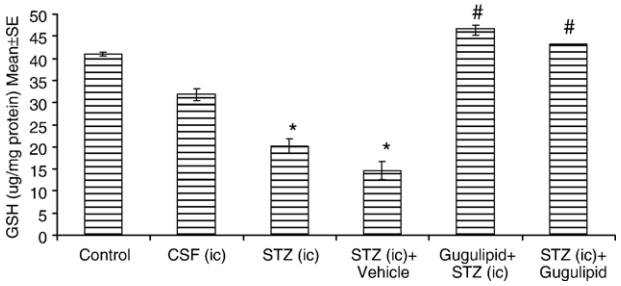

Fig. 7. GSH level in brain of control, artificial CSF (ic), STZ (ic), 1% methyl cellulose in STZ (ic)-treated, (STZ (ic)+vehicle) STZ (ic) in gugulipid pre-treated (Gugulipid+STZ) and gugulipid in STZ (ic)-treated (STZ+Gugulipid) mice. *Significant difference ($p<0.05$) in GSH from control. #Significant difference ($p<0.01$) in GSH from STZ (ic) group; ANOVA (one-way) followed by Tukey test.

3.7.2. Glutathione level

Glutathione was estimated on the 21st day after first dose of STZ. The values of STZ (ic) group, vehicle-treated STZ (ic) group and CSF control group on day 21 were 20.25±3.027, 14.61±2.1 μg/mg and 40.90±4.42, respectively. There was a significant fall in the levels of glutathione in the STZ group and vehicle-treated STZ (ic) group as compared to the CSF control group. STZ (ic) groups treated with gugulipid for 14 days (preventive) and gugulipid-treated STZ (ic) group (post-treatment) showed significant increase in GSH level as compared to the STZ (ic) group[$F(5,30)=20.54$, $p<0.01$, Tukey test]. The values of GSH in STZ (ic) groups in which gugulipid was administered as pre-treatment and post-treatment were 46.74±1.49 and 43.28±2.751 μg/mg proteins respectively (Fig. 7).

3.7.3. Acetyl cholinesterase activity

Acetylcholinesterase activity was estimated on the 21st day after the first dose of STZ. The values of AChE activity in control, STZ (ic) group, vehicle-treated group and CSF control group on day 21 were 0.1320±0.004, 0.2597±0.0023, 0.2635±0.0504 and 0.1212±0.0254 (μmol/min/mg protein) respectively. There was a significant rise in the enzyme activity in the STZ-treated mice and vehicle-treated STZ (ic) mice as

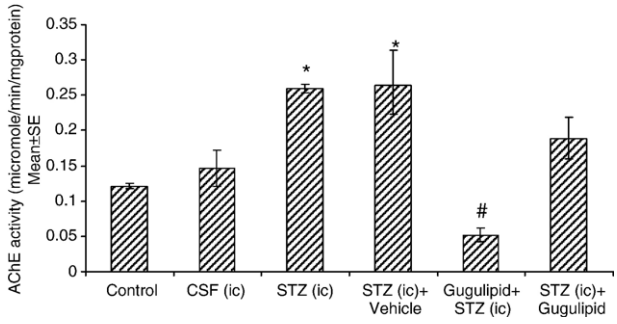

Fig. 8. Comparison of acetylcholinesterase (AChE) activity, in the brain of control, artificial CSF (ic), STZ (ic), 1% methylcellulose in STZ (ic)-treated (STZ (ic)+vehicle), STZ (ic) in gugulipid pre-treated (Gugulipid+STZ) and gugulipid in STZ (ic)-treated (STZ+Gugulipid) mice. *Significant difference ($p<0.01$) from control. #Significant difference ($p<0.01$) in AChE activity from STZ (ic) group; ANOVA (one-way) followed by Tukey test.

compared to that of control group. AChE activity in gugulipid post-treatment in STZ (ic)-treated mice (0.1893±0.0302 μmol/min/mg protein) was not significantly different from STZ and STZ vehicle group. AChE activity in gugulipid pre-treated group was 0.05183±0.0071 μmol/min/mg protein, which is significantly less than that of STZ (ic) and STZ (ic) vehicle-treated groups [$F(5,30)=9.79$, $p<0.01$, Tukey test] (Fig. 8).

3.8. Blood glucose

There was no significant difference in blood sugar level (mg/dl) among control (89.75±5.01), and on 14 days after the first dose of CSF (ic) (83.5±5.62), STZ (ic) (88.66±15.8) and STZ (ic) in gugulipid pre-treated (91.0±13.45) groups [$F(3,20)=0.89$, $p>0.05$, Tukey test].

4. Discussion

Gugulipid, a well-known anti-dyslipidemic and anti-inflammatory drug has shown the potential of an anti-dementia drug in the present study. Gugulipid treatment proffered a salutary effect on rodent models of memory deficits commonly used to screen anti-dementia drugs. The models used were scopolamine or STZ (ic)-induced deficits in passive avoidance and Morris water maze test in mice. Central cholinergic system plays a major role in regulation of cognitive function. A cholinergic neuronal loss in hippocampus is a main characteristic feature of Alzheimer's disease. Scopolamine used in the study to cause memory deficit in passive avoidance test, is a cholinergic muscarinic receptor antagonist and known to cause reversible symptoms of senile dementia in young volunteers (Fibiger et al., 1990; Darren et al., 1992; Prohovnik et al., 1997). Another agent used in the study to produce memory deficit is intracerebral administration of STZ, which causes neuronal damage in the brain by producing free radicals, impairment of glucose utilization and demyelination (Nitsch et. al., 1989; Bastar et al.,1998; Sharma and Gupta, 2001a,b, 2002; Shoham et al., 2003). Twice administration of STZ at 48 h apart in mice by intracerebral route showed a persistent significant deficit in passive avoidance and Morris water maze tests after 14 days of the first dose without altering blood glucose level. Administration of artificial CSF by ic in a manner similar to STZ did not hinder the learning in these tests in mice. These results of STZ memory deficits are in conformity with other workers who have demonstrated cognitive impairment after STZ (icv) in rats (Blokland and Jolles, 1993; Lannert and Hoyer, 1998).

Gugulipid pre-treatment showed a dose related reversal of scopolamine-induced memory deficits as demonstrated by a significant increase in the transfer latency time in retention trial in passive avoidance. The dose of 50 mg/kg, p.o. of gugulipid was significantly effective in increasing the transfer latency time during retention trials. Henceforth, a 50-mg/kg dose of gugulipid was used in subsequent studies. Pre-treatment with gugulipid was also effective in preventing STZ (ic)-induced memory deficit in passive avoidance. To evaluate curative potential of gugulipid, it was administered daily in STZ-treated memory deficient mice and on the third retention trial a significant recovery in memory occurred indicated by an increase in the transfer latency time. Thus, gugulipid showed preventive as well as curative potential for memory impairment in passive avoidance.

Morris water maze is employed to test spatial memory, which is affected severely in Alzheimer's disease. Gugulipid-treated mice showed a better performance in navigating the hidden platform in order to escape from water than control mice. It indicates that gugulipid might enhance memory acquisition and recall in normal situations. The STZ-treated mice did not show the decline in the latency time while control mice showed significant learning on the 3rd session. Pre-treatment (preventive) with gugulipid significantly protected mice from STZ-induced memory impairment. Furthermore, post-treatment (curative) administration of gugulipid in STZ-treated mice resulted into a significant decrease in the latency time indicating a successful restoration of learning and memory functions in erstwhile deficient mice. Gugulipid was also effective in water maze test with visible platform. STZ treatment did not cause a decrease in latency but administration of gugulipid in STZ-treated mice resulted in a significant reduction in latency time in the visible platform test. However, visible platform test is often recommended as non-spatial condition of Morris water maze, but there are reports, which indicate that during visible platform test animals also apply spatial search strategies along with cue learning (Hauben et al., 1999). The results obtained with gugulipid and STZ in this study with visible platform test also support this contention. Sensorimotor effects may influence water maze test with visible or hidden platform (Hooge and De Deyn, 2001). The locomotor activity of control, gugulipid and STZ (ic)-treated group showed no significant difference, which excludes the possibility of interference by locomotor activity in Morris water maze and passive avoidance tests in STZ (ic) mice and the gugulipid-treated STZ (ic) group. Therefore, gugulipid showed its efficacy as preventive and curative against STZ model of dementia in both passive avoidance and Morris water maze test.

Alzheimer's disease is one of the most common forms of neurodegenerative disorder characterized by a progressive loss of memory, followed by a complete dementia (Yankner, 1996). Several studies suggest that oxidative stress plays an important role in pathogenesis of neurodegenerative disorder like Alzheimer's disease (Simonian and Coyle, 1996; Zeevalk et al., 1998; Geula, 1998; Marksbery, 1997). Thus, the progression of neurodegenerative disorder can be inhibited by the use of free radical scavangers and anti-oxidants (Sharma and Gupta, 2001a,b). MDA is an important marker for lipid peroxidation and GSH activity is an indicator of free radical generation. STZ neurotoxicity is attributed to free radical generation (Sharma and Gupta, 2001a,b). In the present study, MDA and GSH were estimated on the 21st day after the 1st injection of STZ because a maximum change in these biochemical parameters is reported to occur on the 21st day (Sharma and Gupta, 2001a,b). STZ treatment showed an increase in MDA and a decrease in GSH level suggesting an increased generation of free radicals. The chronic treatment of gugulipid in STZ (ic)-treated mice produced a significant fall in MDA levels in comparison to

that of vehicle-treated mice. Furthermore, there was a significant increase in the levels of GSH in the brain of gugulipid-treated STZ (ic) mice as compared to the vehicle-treated STZ (ic) mice indicating the inhibition of oxidative stress in the brain by gugulipid. Anti-oxidant property of gugulipid is also reported by other workers. Results from our studies and also others (Chander et al., 2002) suggest the anti-oxidant property of gugulipid.

In several neurodegenerative diseases neuronal injury itself can induce free radical generation, which initiate a cascade of events that can lead to neuronal death (Marksbery, 1997; Gupta et al., 1999) which suggests that therapeutic efforts aimed at removal of free radicals or prevention of their formation may be beneficial in diseases like Alzheimer's disease. Gugulipid has been found in this study to be effective in reducing oxidative damage in the central nervous system.

The cognitive deficit associated with AD is suggested to be primarily related to defects of cholinergic neurotransmission in the brain. The inhibition of AChE, metabolizing enzyme of acetylcholine, in the brain is important for increasing cholinergic neurotransmission. Therefore, use of the cholinesterase inhibitors is the most effective pharmacological approach for the symptomatic treatment of AD (Racchi et al., 2004). Gugulipid also showed inhibition of AChE activity in brain of STZ (ic)-treated mice but its inhibitory effect on AChE was significant only when it was administered for 14 days (pre-treatment) as compared to its administration for 5 days (post-treatment). This finding indicates that chronic treatment of gugulipid may also enhance cholinergic activity another favorable factor for cognitive improvement by inhibition of AChE.

Besides it, data from epidemiological studies and animal models imply that disturbances in cholesterol metabolism are linked to Alzheimer's disease susceptibility. Many observational studies make it evident that patients receiving statins have a reduced risk of dementia (Jick et al., 2000). Lipid-lowering agents may have implications for the prevention of Alzheimer's disease. Gugulipid is principally a lipid-lowering agent. Indeed, in this study gugulipid showed a significant protective role against STZ (ic)-induced cognitive impairment as well as oxidative stress.

In conclusion, the present study clearly demonstrates that gugulipid significantly prevented and restored the cognitive impairment that can be attributed to the attenuation of the oxidative stress and enhancement of cholinergic influence. With the addition of anti-dementia activity obtained in this study to already reported effects of gugulipid — anti-dyslipidemic, anti-diabetic and anti-inflammatory, gugulipid emerges as an important potential drug for age related neurodegenerative disorders where oxidative stress and cognitive impairment are involved.

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