Ind. J. Pharm. Edu. Res., 2024; 58(2):503-509.
https://www.ijper.org

Original Article

Danshensu Attenuates Diabetes Associated Cognitive Dysfunction by Markedly Reversing Oxidative Stress, Aβ and AChE Activity

Sunil Shukla[1,*], Sunil Sharma[1], Neeru Vasudeva[1], Tanuj Hooda[2,*]

[1]Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar, Haryana, INDIA.
[2]M. M. College of Pharmacy, Maharishi Markandeshwar (Deemed to be University) Mullana, Ambala, Haryana, INDIA.

ABSTRACT

Background: Evidences suggest that the development of neurodegenerative dementia is accelerated by Diabetes Mellitus (DM), a chronic metabolic condition. In Diabetes Mellitus (DM), improper glucose metabolism produces glycotoxins that cause beta-amyloid peptides to build up and cause oxidative damage to neurons in the brain tissue. **Aim:** This investigation aims to look at the neuroprotective benefits of Danshensu on memory. **Materials and Methods:** Involving the behaviour model (Morris water maze) and biochemical activities (measurement of glucose, $A\beta_{(1-42)}$ levels, AChE, MDA, and GSH) for acute as well as chronic conditions in STZ-induced diabetic rats at 2 doses of Danshensu namely 2 mg/kg and 5 mg/kg body weight per day. **Results:** Results demonstrated an improvement in learning ability in both acute and chronic level treatment. Additionally, there was an alteration in the levels of biochemical markers after treatment with Danshensu, indicating that this substance may influence transmission of cholinergic neurons and potentially alleviate cognitive impairments brought on by oxidative damage. **Conclusion:** Danshensu ameliorates the effects of STZ more effectively at a dose of 5 mg/kg rather than 2 mg/kg at the acute level of treatment whereas at a chronic level, both 2 mg/kg and 5 mg/kg doses demonstrate an equally significant result. Therefore, Danshensu is a potential candidate for treating hyperglycemia-induced cognitive dysfunction.

Keywords: Diabetes, Alzheimer's disease, Oxidative stress, Danshensu, Beta-amyloid, AChE activity.

Correspondence:
Dr. Tanuj Hooda
M. M. College of Pharmacy, Maharishi Markandeshwar (Deemed to be University) Mullana, Ambala, Haryana, INDIA.
Email: tanujhooda2010@gmail.com.

Sunil Shukla,
Research Scholar, Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar- 125001, Haryana, INDIA.
Email: sun.shukla5@gmail.com

Received: 03-09-2023;
Revised: 28-11-2023;
Accepted: 12-12-2023.

INTRODUCTION

Diabetes Mellitus (DM) refers to a chronic metabolic disorder that is spreading alarmingly around the world with Diabetes Mellitus Type 2 (T2DM), a subtype comprising 95% of this disease.[1,2] T2DM is mainly characterized by a deficit in insulin actions and signalling. Hyperglycaemia and hyperinsulinemia are caused by insulin resistance in peripheral organs. Hepatocytes and pancreatic cells are damaged by glucose toxicity due to endoplasmic reticulum stress, oxidative stress, and mitochondrial dysfunction. Additionally, long-term exposure to hyperglycaemia might impair cognitive performance. This Hyperglycaemia-induced cognitive disturbance is regarded as a brain consequence of diabetes.[3]

However, Alzheimer's Disease (AD) is a widespread neurological disorder, where patients suffer from a slow loss of cognitive skills and memory making it difficult to even carry out daily basic needs.[4] The presence of beta-Amyloid (Aβ), neuronal loss, and neurofibrillary tangles are the three main pathogenic traits of AD. Existing data shows that oxidative stress is a major factor in the onset and progression of AD.[5] Moreover, evidence supports that DM is responsible for the progression of neurodegenerative dementia. In DM, cognitive dysfunction and dementia are mediated by the glycotoxins or advanced glycation end products produced by abnormal glucose metabolism, these toxic products lead to the accumulation of beta-amyloid peptides and oxidative damage of neurons in brain tissue.[6,7] The Hippocampus and cortex are the main regions of brain for cholinergic transmission and cover the cognitive function and memory that seems to be more prone to oxidative damage and lead to impairment of the normal function of cholinergic neurons, which may contribute to cognitive dysfunction. Antioxidants and glucose lowering agents mainly that increase insulin sensitivity are reported to ameliorate cognitive dysfunction in DM.[8] However, here is no specific strategy that can be used for the prevention and management of cognitive impairment in Diabetes.

DOI: 10.5530/ijper.58.2.56

Danshensu is a phenolic compound of plant origin *Salvia miltiorrhiza* that possesses anti-oxidant properties, and can protect neurons in brain tissue from oxidative injury.[9,10] The aim of the current research is to examine the neuroprotective role of Danshensu on memory involving the behaviour model (Morris water maze) and biochemical activities (measurement of glucose, Aβ, AChE, MDA, and GSH) for acute and chronic conditions in STZ-induced diabetic model of rats at 2 doses namely 2 mg and 5 mg/kg body weight/day.

MATERIALS AND METHODS

Animals

Wistar rats (180-220 g) were obtained from the Lala Lajpat Rai University of Veterinary and Animal Sciences' infection free small animal facility in Hisar (Haryana, India). Rats were kept in a space with a 12 hr light/dark cycle and standard laboratory temperature and humidity, with unrestricted access to both food and water. The acclimation period for animals was one week, carried out before the experimental work. All scientific procedures adopted with animals as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India. The Institutional Animal Ethics Committee authorised the study design at its meeting on 6 October 2017 (Endst no. of minutes of IAEC meetings-IAEC/2017/44-52).

Chemicals

Danshensu (Danshensu sodium salt) was procured from Sigma Aldrich (Merk). Donepezil was procured from Cipla, India and STZ from SRL and Acetylthiocholine iodide were procured from Sigma Aldrich (Merk). $A\beta_{(1-42)}$ kit was bought from Genxbio Health Sciences Pvt Ltd. Prior to administration, all drug solutions were freshly prepared.

In vivo experimental design

All 60 rats were fed in an adaptive manner for 7 days, 12 rats were provided basic food as a standard control (Normal). Streptozotocin (STZ) 60 mg/kg Body Weight (BW) was injected through intraperitoneal (i.p.) to induce diabetes in the rats.[11] Blood was collected from the tail vein 72 hr after the injection, and blood glucose levels was assessed. If the diabetic models' blood sugar levels were greater than 280 mg/dl, they were selected for the study. The remaining 48 animals with diabetes were divided randomly into eight groups (n=6/group), four each for checking acute as well as chronic conditions of treatment. The groups assigned are as follows: Diabetic rats without any treatment (STZ diabetic group); Diabetic rats treated with Donepezil which is used as positive standard.[12] Diabetic rats treated with Danshensu at dose of 2 mg/kg and 5 mg/kg respectively.

These established diabetic rat models were kept for 4 weeks in order to develop persistent hyperglycaemia and treatment was started on 39th day by injecting Danshensu (Danshensu sodium salt) 2 mg/kg and 5 mg/kg BW i.p. for 14 days in case of acute treatment and for 28 days in case of chronic treatment in order to evaluate the effect of time and dosing in the management of AD. At the same time Alzheimer diabetic rats' model were treated with Donepezil (5 mg/kg p.o.) for 14 and 28 days, respectively.

Morris Water Maze (MWM)

The MWM test, which gauges learning and memory capacity, was used to gauge escape delay. The experiment was carried out in a circular pool, approximately 200 cm diameter with water kept at a temperature of 25°C±2°C. In the middle of the quadrant, place the rectangular platform (diameter: 10 cm), 2 cm below the level of water and made the water opaque with dye to hidden the platform. There are four quadrants in the pool, each with identical spacing and marked the quadrants as 1, 2, 3 and 4. Experiment was started with quadrant 1 and followed by remaining three quadrants. For four days (once per day), rats underwent training sessions that could last no more than 120 sec in each of the four quadrants. Measurements were made on the amount of time it took to reach the hidden platform (escape latency). A rat was directed onto the platform (where it was held for 20 s) if rat didn't reach the platform within 120 sec, and the latency was then captured as 120 sec. On day 5 of the probing trial, the platform was taken away, and the animals were given 120 sec to look for it. The time spent in the target quadrant and the frequency of platform crossings were noted.[13]

Biochemical estimations

Glucose estimation

All rats underwent blood glucose test with a portable glucometer (Accu-Check), utilising tail vein blood to analyse the blood glucose level after 14 and 28 days of treatments.

Brain tissue preparation

After 14 and 28 days of treatments, the animals were sacrificed by cervical dislocation in order to isolate the brain tissues. A glass Wise Stir Homogenizer was used to homogenize the entire brain after it had been carefully removed from the skull and placed in 0.1 M phosphate-buffered saline (pH 7.4). After centrifuging the brain tissue homogenates at 10000 g for 10 min at 4°C the supernatant was subsequently stored at -80°C for use in various biochemical investigations.

Estimation of protein

By employing the Erba Liquixx protein estimation kit, which is based on the Biuret Method, total protein in various rat brain structures was determined.

Measurement of Aβ

Measurement of $A\beta_{(1-42)}$ was done by using beta-Amyloid (1-42) ELISA Kit (Genxbio Health Sciences Pvt. Ltd.,) in accordance with the manufacturer's instructions. The $A\beta_{(1-42)}$ level was measured in pg/mL.

Acetylcholinesterase (AChE) activity

The spectrophotometric method of Ellman et al. (1961) was adopted to determine the AChE enzymatic activity with some modification.[14,15] The reaction mixture (3 mL total volume) comprised 0.1 mL sample, 0.2 mL 5,5′-Dithiobisnitrobenzoic acid (DTNB), 0.2 mL acetylthiocholine iodide (75 mM) and 2.5 mL of potassium phosphate buffer, pH 7.5. The procedure relies on the creation of the 5,5′-dithio-bis-acidnitrobenzoic yellow anion, which is detected by absorbance at 412 nm after a 2 min incubation at 25°C. A change in optical density was noted at an interval of 30 s, for 2 min. The AChE activity was measured in nmoles of acetylthiocholine iodide hydrolysed/min/mg of protein.

Estimation of malondialdehyde (MDA)

Lipid peroxidation levels in the rat's brain were measured, as reported earlier.[16] 0.5 mL of tissue homogenate and 0.5 mL of Tris HCl (pH 7.4) were incubated at 37°C for around two hr. After that, 1 mL of TCA (10%) was combined and spun at a speed of 1000 g for 10 min. Finally, after addition of 1 mL of thiobarbituric acid (0.67%), the solution was maintained in a bath of boiling water for 1 hour, and the MDA levels was determined by reaction with TBA at 532 nm using UV-vis spectrophotometry. The results were expressed is nmoles/mg protein.

Estimation of reduce Glutathione (GSH)

GSH levels in the brain tissue homogenate were estimated using, Ellman method.[17] A mixture made up of equal quantity of supernatant and sulfosalicylic acid (4%) and kept at 4°C for an hour. Following that, the sample was centrifuged at 1200 g for almost 5 min at 4°C. 0.2 mL DTNB and 2.7 mL of 0.1 M phosphate buffer (pH 8) were then added to the centrifuged supernatant. The optical density was measured at wavelength of 412 nm, and the results were expressed in nmoles of GSH/mg protein.

Statistical analysis

For all statistical calculations, GraphPad Prism (GraphPad Software, San Diego, CA) was utilized. The mean±SEM is used to express values. A repeated measure two-way analysis of variance (ANOVA) was used to examine the escape latency (behavioural assessment data), with drug-treated groups acting as both within-subjects and between-sessions factors. The one-way ANOVA was used to assess the other behavioural assessment and biochemical estimates. Using Tukey's test, *post hoc* comparisons between groups were done. *P* values under 0.05 were deemed significant.

RESULTS

Morris water maze

In this behavioural test, animals were subjected for training for four days to find hidden platform after 14 and 28 days of treatment. All animal groups shown inadequate learning capacity on the first day, and no significant variations in escape latency were found between any animal groups. From day 2 to day 4 of training, all animal groups showed significant improvement in learning ability but STZ infused animals group demonstrated poor learning ability ($p<0.001$) in comparison to the control group of rats. After 14 days of treatment, animal groups treated with Danshensu showed dose dependent improvement in learning behaviour during the training *vis-a-vis* STZ infused group. As it is evident from results that there was significant improvement in learning, i.e. Danshensu 2 mg ($p<0.01$) and Danshensu 5 mg ($p<0.001$) when compared with the STZ infused group as depicted in Figure 1 (A). However, after 28 days of treatment, both doses of Danshensu showed equally significant ($p<0.001$) in improvement of learning ability when compared to the STZ infused group. For reference memory, probe trial test was also performed, and results demonstrated that STZ infused group showed significant decrease in % dwell time in comparison to the control and Donepezil treated groups in both 14 and 28 days of study. However, after 14 days of treatment it was observed that animal groups treated with Danshensu 2mg and 5mg showed significant increase ($p<0.05$) in % dwell time as shown in Figure 1(B), whereas there was dose dependent increment in the % dwell time at chronic level. Moreover, when compared to the diabetic (STZ) group, Danshensu 5 mg and Danshensu 2 mg ($p<0.01$, $p<0.05$) treated group demonstrated a significant increment in % dwell time as depicted in Figure 1(B). Furthermore, administration of STZ led to a significant decrement in the platform crossing frequency in comparison to the control group for both 14 and 28 days of study. But results notably specified that animal groups treated with Danshensu 2 mg and 5 mg showed equally significant improvement ($p<0.05$) in platform crossing frequency *vis-à-vis* STZ infused group post 14 days of treatment. However, Danshensu treated groups illustrated a dose dependent increment in crossing frequency post 28 days of treatment *vis-a-vis* STZ infused group as depicted in Figure 1 (C).

Glucose

The increased glucose level in rats causes memory impairment, therefore STZ-infused animal group showed upregulation ($p<0.001$) in the glucose levels as compared to the control group in both acute and chronic level of the study. However, rats treated with Danshensu 2 mg and 5 mg doses showed significant ($p<0.001$) reduction in glucose levels in both 14 days and 28 days treatment studies illustrated in Figure 2.

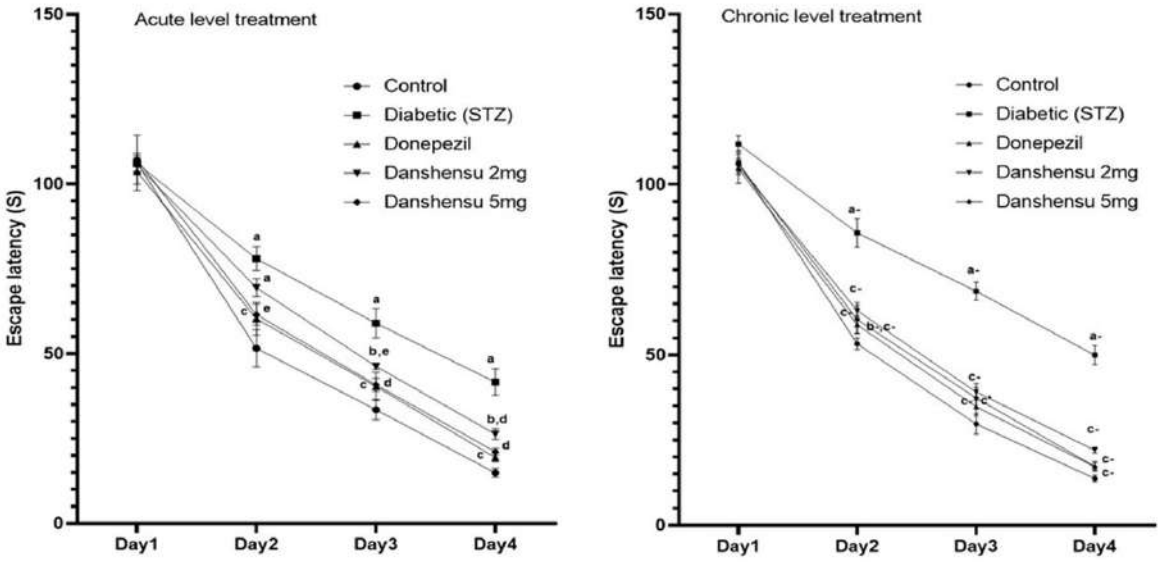

Figure 1 (A)

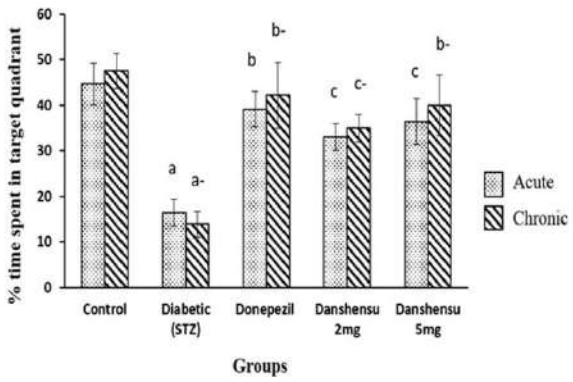

Figure 1 (B)

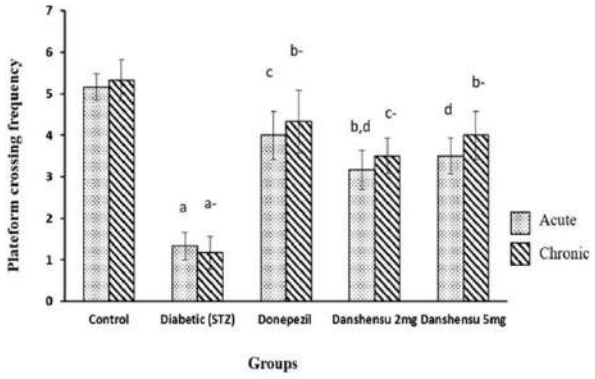

Figure 1 (C)

Figure 1: (A): Effect of Danshensu on escape latency at acute as well as chronic levels. The escape latencies are expressed in mean±SEM. For acute level study results are a (p<0.001) vs Control; b (p<0.05) vs Control; c (p<0.001) in comparison to STZ; d (p<0.01) vs STZ; e (p<0.05) vs STZ; whereas for chronic level study results are a-p<0.001 vs Control; b-(p<0.05) vs Control; c-(p<0.001) in comparison to STZ. Figure 1 (B): Impact of Danshensu on % time spent in the intended quadrant in MWM at acute as well as chronic levels. This figure depicts the consequence of Diabetes mellitus on learning ability in STZ injected rats. The drug treated rat showed significant improvement in learning ability in both acute and chronic level treatment. After acute level treatment, animal groups treated with Danshensu showed dose dependent improvement in learning behaviour however, after chronic level treatment, both doses of Danshensu showed equally significant results in learning ability. The % time spent in target quadrant is demonstrated as mean±SEM. For acute level study results are a (p<0.001) vs Control; b (p<0.01) vs STZ; c (p<0.05) in comparison to STZ; whereas for chronic level study results are a-(p<0.001) vs Control; b-(p<0.01) against STZ; c-(p<0.05) vs to STZ. Figure 1 (C): Impact of Danshensu on platform crossing frequency in Morris water maze at acute as well as chronic levels. This figure illustrates the consequences of Diabetes mellitus on learning behaviour in STZ injected rats. The drug treated rat showed significant improvement in learning ability in both acute and chronic level treatment. Acute level treatment showed equally significant improvement in platform crossing frequency at both the doses. However, Danshensu treated groups illustrated a dose dependent increment in crossing frequency at chronic level. The values of time spent in target quadrant are depicted as mean±SEM. For acute level study results are a (p<0.001) vs Control; b (p<0.05) vs Control; c (p<0.01) in comparison to STZ and d (p<0.05) vs STZ whereas for chronic level study results are a-(p<0.001) vs Control; b-(p<0.01) as in comparison to STZ; c-(p<0.05) vs STZ.

$A\beta_{(1-42)}$ level

Administration of STZ demonstrated a substantial rise in the intensity of $A\beta_{(1-42)}$ in comparison to the control group of animals. However, after 14 days of treatment with Danshensu results demonstrated a dose dependent decrement in $A\beta_{(1-42)}$ concentration in rat's brain in comparison to STZ group. Similarly, the animals group administered with Danshesu 2 mg and 5 mg for 28 days showed equally significant (p<0.001) reduction in $A\beta_{(1-42)}$ concentration *vis-à-vis* STZ group as depicted in Figure 3.

AChE activity

Animals infused with STZ exhibited notable upregulation in AchE enzyme activity in rat's brain as compared to the control group. However, rats treated with Danshensu for 14 days

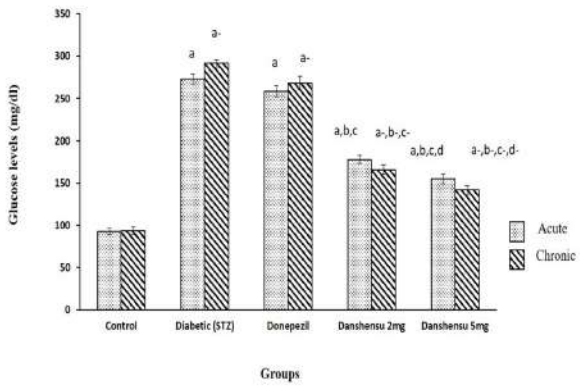

Figure 2: Impact of Danshensu on blood glucose levels at acute and chronic level treatment. The figure shows the effect of different doses of Danshensu on blood glucose levels. Animals treated with Danshensu 2 mg and 5 mg doses showed a significant reduction in glucose levels in both acute and chronic treatment studies. The values are depicted as mean±SEM. For acute level study results are a (p<0.001) vs Control; b (p<0.001) vs STZ; c (p<0.001) in comparison to Donepezil and d (p<0.05) against Danshensu 2 mg; whereas for chronic level study results are a-(p<0.001) vs Control; b-(p<0.001) vs STZ; c-(p<0.001) when compared to Donepezil and d- (p<0.05) vs Danshensu 2 mg.

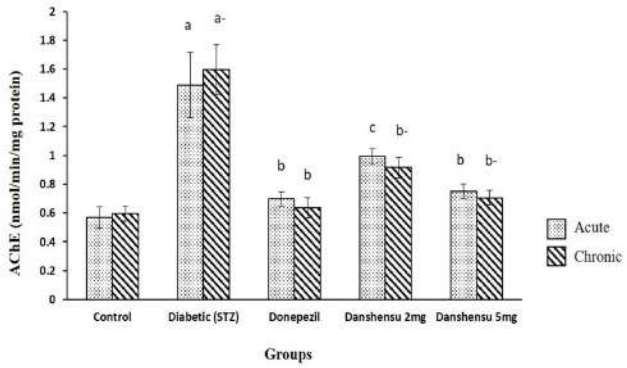

Figure 4: AChE activity in the brain of rats with diabetes caused by STZ and those receiving Danshensu. The figure illustrates the effect of Danshensu on AChE enzyme activity of brain tissue in STZ injected rats. At acute level of treatment with Danshensu results demonstrated a dose dependent decrement in AChE enzyme activity in rat's brain. Similarly, animals group treated with Danshensu 2 mg and 5 mg chronically showed an equal significant reduction in AChE activity. The values are depicted as mean±SEM. For acute level study results are a (p<0.001) vs Control; b (p<0.001) vs STZ and c (p<0.05) when compared to STZ; whereas for chronic level study results are a-(p<0.001) vs Control; b-(p<0.001) vs STZ.

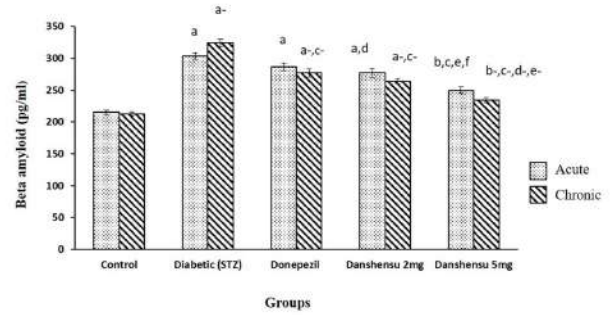

Figure 3: $A\beta_{(1-42)}$ levels in the brain of rats with diabetes caused by STZ and those receiving Danshensu treatment. The figure shows the effect of Danshensu on $A\beta_{(1-42)}$ level activity of brain tissue in STZ injected rats. At acute level of treatment with Danshensu results demonstrated a dose dependent decrement in $A\beta_{(1-42)}$ concentration in rat's brain. Similarly, animals group treated with Danshesu 2 mg and 5 mg chronically showed equally reduction in $A\beta_{(1-42)}$ concentration. The values are depicted as mean±SEM. For acute level study results are a (p<0.001) vs Control; b (p<0.01) vs Control; c (p<0.001) vs STZ; d (p<0.05) vs STZ; e (p<0.001) when compared to Donepezil and f (p<0.05) vs Danshensu 2 mg; whereas for chronic level study results are a-(p<0.001) vs Control; b-(p<0.05) when compared to Control; c-(p<0.001) vs STZ; d-(p<0.001) vs Donepezil; e-(p<0.01) vs Danshensu 2 mg.

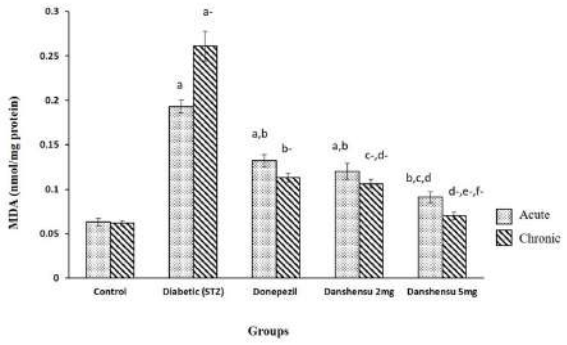

Figure 5: MDA levels in the brain of diabetic rats and those treated with Danshensu. The figure shows the effect of Danshensu on MDA level of brain tissue in STZ injected rats. Animal groups treated with Danshensu 2 mg and Danshensu 5 mg showed a significant decline in MDA level vis-à-vis STZ infused group for both acute and chronic levels of study. The values are depicted as mean±SEM. For acute level study results are a (p<0.001) vs Control; b (p<0.001) vs STZ; c (p<0.01) vs Donepezil; d (p<0.05) vs Danshensu 2 mg; whereas for chronic level study results are a-(p<0.001) vs Control; b-(p<0.01) vs Control; c-(p<0.05) vs Control; d-(p<0.001) when compared to STZ and e- (p<0.05) vs Donepezil; f-(p<0.05) vs Danshensu 2 mg.

displayed dose dependent downregulation in AChE enzyme activity as compared to the STZ group as shown in Figure 4. However, after 28 days of treatment with Danshensu 2 mg and 5 mg doses, results demonstrated a similar substantial reduction in AChE enzyme activity (p<0.001) as compared to the STZ infused group.

MDA level

MDA is a marker indicator for oxidative damage in tissue. A substantial rise in the brain MDA level was observed in STZ infused rats' *vis-à-vis* control group. Whereas animal group treated with Danshensu 2 mg and Danshensu 5 mg demonstrate a marked decline in brain MDA against the STZ infused group for both 14 and 28 days of study Figure 5.

GSH level

A significant downregulation was observed in GSH level after administration of STZ *vis-à-vis* the control group. However, animal groups treated with Danshensu 2 mg and 5 mg for 14 days

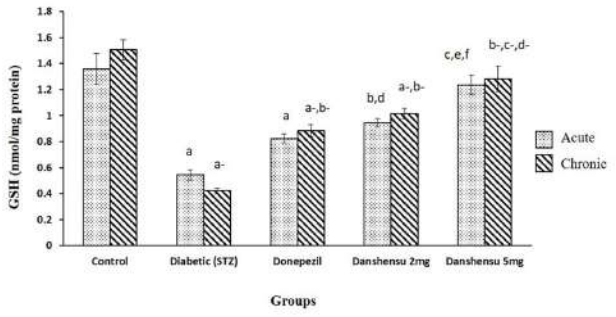

Figure 6: GSH levels in brain of rats with diabetes caused by STZ and those treated with Danshensu. The figure shows the effect of Danshensu on GSH levels of brain tissue in STZ administered rats. Animal groups treated with Danshensu 2 mg and Danshensu 5 mg showed a significant upregulation in GSH levels as compared to the STZ infused group for the both acute and chronic levels of study. The values are depicted as mean±SEM. For acute level study results are a (p<0.001) vs Control; b (p<0.01) vs Control; c (p<0.001) vs STZ; d (p<0.01) vs to STZ and e (p<0.01) as compared to Donepezil; f (p<0.05) vs Danshensu 2 mg; whereas for chronic level study results are a-(p<0.001) vs Control; b-(p<0.001) vs STZ; c- (p<0.01) vs Donepezil; d-(p<0.05) vs Danshensu 2 mg.

and 28 days demonstrated significant upregulation (p<0.001) in GSH levels against the STZ administered group Figure 6.

DISCUSSION

DM may be either due to insufficient insulin secretion by the beta cells of the pancreas or due to insulin resistance in the body.[6] Diabetes is linked to cognitive dysfunctions that are accompanied by structural and neurophysiological alterations in the CNS.[18]

In the present study, MWM technique was performed for memory and learning ability in rats. Animals infused with STZ showed a significant increment in escape latency throughout the training period and a reduction in % dwell time in the target quadrant throughout the probe trial similar to the one reported in the previous studies.[19] However, treatment with Danshensu 2 mg/kg and 5 mg/kg BW respectively showed dose dependent improvement in learning and recollection of memory in contrast to the diabetic (STZ infused) group in both acquisition and probe trials. This decline in poor learning ability post-treatment could be attributed to the antioxidant potential of Danshensu.

Increase in Amyloid Precursor Protein (APP) expression and Aβ deposition, as are present in DM, play a vital role in genesis of senile plaques in rats brain tissue. Build-up of $A\beta_{(1-42)}$ is more rapid as compared to $A\beta_{(1-40)}$ in brain tissue. Also, $A\beta_{(1-42)}$ is more toxic than $A\beta_{(1-40)}$.[20] In this study, $A\beta_{(1-42)}$ concentration was determined in rats' brain using ELISA. In studies at both the acute and chronic level, animals given STZ revealed significantly higher levels of $A\beta_{(1-42)}$ in rats brain tissue as compared to control group. However, animal groups treated with Danshensu showed dose dependent decrement in the $A\beta_{(1-42)}$ level *vis-à-vis* STZ administered group after 14 days of treatment, whereas after 28 days of treatment with Danshensu 2 mg and 5 mg showed equally significant downregulation of $A\beta_{(1-42)}$ levels against STZ infused group. This suggests that, for rapid reversal of $A\beta_{(1-42)}$ burden in brain tissue, Danshensu 5 mg dose is more effective as compared to Danshensu 2 mg. The attenuation of $A\beta_{(1-42)}$ levels by Danshensu may be due to its anti-oxidative potential as mentioned earlier.[21]

Previous findings suggest that diabetes mellitus increases Acetylcholinesterase (AChE) activity, which may cause the dysfunction of cholinergic neurotransmission in the brain and this may lead to cognitive impairment.[22] Increased levels of AChE lead to rapid hydrolysis of acetylcholine, a neurotransmitter that participates in cognitive function.[23] In the current study, the STZ-infused group had higher AChE activity than the control group.[24] However, the animal group treated with Danshensu 2 mg and 5 mg doses for 14 days showed dose dependent inhibition of AChE activity, but after 28 days of treatment, the study revealed that both doses of Danshensu was equally significant in inhibiting AChE activity. A positive control group (Donepezil) was somewhat more effective in inhibition of AChE activity as compared to the groups treated with Danshensu.

In DM, prolong increase in glucose level in brain is the main mediator for the genesis of Reactive Oxygen Species (ROS) leading to oxidative stress.[25] Oxidative stress plays important role in pathophysiology of Alzheimer disease (AD) and causes cognitive impairment.[26] Increased lipid peroxidation and a decrease in antioxidant enzyme activity are two often used indicators of oxidative stress. Prior studies on the post-mortem brains of AD patients showed elevated levels of protein and lipid oxidation.[27] In the present study, administration of STZ in Wistar rats results in increased lipid peroxidation and decreased GSH levels. Danshensu, recognized for its antioxidant activity, considerably reversed the harmful effects of STZ on brain MDA and GSH levels. This observation indicates that Danshensu exerts a defensive mechanism against oxidative stress and play important role in the reversal of memory and learning ability. Thus, Danshensu may be a potential candidate for reversal of DM induced cognitive impairment.

CONCLUSION

The present study demonstrates a decline in poor learning ability post-treatment which could be attributed to the antioxidant potential of Danshensu. Moreover, for the rapid reversal of $A\beta_{(1-42)}$ burden in brain tissue, Danshensu 5 mg dose is more effective as compared to Danshensu 2 mg. Additionally increased AChE activity and lipid peroxidation as well as decreased GSH in the brain tissue of STZ infused diabetic rats were altered (downregulation of AChE and LPO as well as upregulation in GSH) after treatment with Danshensu, indicating that this substance may modulate cognitive function that are altered by oxidative stress. However, it was observed that Danshensu ameliorates the effects of STZ more effectively at a dose of 5 mg

rather than 2 mg at the acute level of treatment whereas at a chronic level both 2 mg and 5 mg doses demonstrate an equally significant result. As a result, we may infer that Danshensu is a potential natural compound with significant neuroprotective properties that needs to be researched more in order to develop a more effective treatment for individuals with memory problems brought on by the hyperglycaemic state.

ACKNOWLEDGEMENT

The authors wish to express their sincere gratitude to the Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar for providing them with all the resources needed for the study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

AD: Alzheimer's disease; **Aβ:** Amyloid β peptide; **APP:** Amyloid precursor protein.; **AChE:** Acetylcholinesterase; **ANOVA:** Analysis of variance; **BW:** Body weight; **CNS:** Central nervous system; **DM:** Diabetes mellitus; **DTNB:** 5,5′-dithiobisnitrobenzoic acid; **ELISA:** Enzyme linked immunosorbent assay; **GSH:** Glutathione; **i.p.:** Intraperitoneally; **LPO:** Lipid peroxidation; **MDA:** Malondialdehyde; **MWM:** Morris water maze; **PCF:** platform crossing frequency; **ROS:** Reactive oxygen species; **STZ:** Streptozotocin; **T2DM:** Type 2 diabetes mellitus; **TCA:** Trichloro acetic acid; **TBA:** Thiobarbituric acid

REFERENCES

1. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2009; 32(1):S62-7.
2. Taylor R. Insulin resistance and type 2 diabetes. Diabetes. 2012;61(4):778-9. doi: 10 .2337/db12-0073.
3. Lee HJ, Seo HI, Cha HY, Yang YJ, Kwon SH, Yang SJ. Diabetes and Alzheimer's disease: mechanisms and nutritional aspects. Clin Nutr Res. 2018;7(4):229-40. doi: 10.7762/c nr.2018.7.4.229, PMID 30406052.
4. Chatterjee S, Mudher A. Alzheimer's disease and type 2 diabetes: a critical assessment of the shared pathological traits. Front Neurosci. 2018;12:383. doi: 10.3389/fnins.201 8.00383, PMID 29950970.
5. Zhao Y, Zhao B. Oxidative stress and the pathogenesis of Alzheimer's disease. Oxid Med Cell Longev. 2013;2013:316523. doi: 10.1155/2013/316523, PMID 23983897.
6. Saini V. Molecular mechanisms of insulin resistance in type 2 diabetes mellitus. World J Diabetes. 2010;1(3):68-75. doi: 10.4239/wjd.v1.i3.68, PMID 21537430.
7. Butterfield DA, Di Domenico F, Barone E. Elevated risk of type 2 diabetes for development of Alzheimer disease: a key role for oxidative stress in brain. Biochim Biophys Acta. 2014;1842(9):1693-706. doi: 10.1016/j.bbadis.2014.06.010, PMID 24949886.
8. Maciel RM, Carvalho FB, Olabiyi AA, Schmatz R, Gutierres JM, Stefanello N, *et al.* Neuroprotective effects of quercetin on memory and anxiogenic-like behavior in diabetic rats: role of ectonucleotidases and acetylcholinesterase activities. Biomed Pharmacother. 2016; 84: 559-68. doi: 10.1016/j.biopha.2016.09.069, PMID 27694000.
9. Tang Y, Wang M, Le X, Meng J, Huang L, Yu P, *et al.* Antioxidant and cardioprotective effects of danshensu (3-(3, 4-dihydroxyphenyl)-2-hydroxy-propanoic acid from *Salvia miltiorrhiza*) on isoproterenol-induced myocardial hypertrophy in rats. Phytomedicine. 2011; 18(12): 1024-30. doi: 10.1016/j.phymed.2011.05.007, PMID 21665454.
10. Seetapun S, Yaoling J, Wang Y, Zhu YZ. Neuroprotective effect of danshensu derivatives as anti-ischaemia agents on SH-SY5Y cells and rat brain. Biosci Rep. 2013; 33(4). doi: 10.1042/BSR20130032, PMID 23869993.
11. Baluchnejadmojarad T, Kiasalari Z, Afshin-Majd S, Ghasemi Z, Roghani M. S-allyl cysteine ameliorates cognitive deficits in streptozotocin-diabetic rats via suppression of oxidative stress, inflammation, and acetylcholinesterase. Eur J Pharmacol. 2017; 794: 69-76. doi: 10.1016/j.ejphar.2016.11.033, PMID 27887948.
12. Cacabelos R. Donepezil in Alzheimer's disease: from conventional trials to pharmacogenetics. Neuropsychiatr Dis Treat. 2007; 3(3): 303-33. PMID 19300564.
13. Tian Z, Wang J, Xu M, Wang Y, Zhang M, Zhou Y. Resveratrol improves cognitive impairment by regulating apoptosis and synaptic plasticity in streptozotocin-induced diabetic rats. Cell Physiol Biochem. 2016; 40(6): 1670-7. doi: 10.1159/000453216, PMID 28006780.
14. Ellman GL, Courtney KD, Andres Jr V, Feather-stone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961; 7(2): 88-95. doi: 10.1016/0006-2952(61)90145-9, PMID 13726518.
15. Rocha JBT, Emanuelli T, Pereira ME. Effects of early undernutrition on kinetic parameters of brain acetylcholinesterase from adult rats. Acta Neurobiol Exp (Wars). 1993; 53(3): 431-7. PMID 8249659.
16. Wills ED. Mechanisms of lipid peroxide formation in animal tissues. Biochem J. 1966; 99(3): 667-76. doi: 10.1042/bj0990667, PMID 5964963.
17. Ellman GL. Tissue sulphydryl groups. Arch Biochem Biophys. 1959;82:70-7.
18. Zhang J, Liu Z, Li Z, Wang Y, Chen Y, Li X, *et al.* Disrupted white matter network and cognitive decline in type 2 diabetes patients. J Alzheimers Dis. 2016;53(1):185-95. doi: 10.3233/JAD-160111, PMID 27163818.
19. Zou W, Yuan J, Tang ZJ, Wei HJ, Zhu WW, Zhang P, *et al.* Hydrogen sulfide ameliorates cognitive dysfunction in streptozotocin-induced diabetic rats: involving suppression in hippocampal endoplasmic reticulum stress. Oncotarget. 2017;8(38):64203-16. doi: 10.18632/oncotarget.19448, PMID 28969063.
20. El-Agnaf OM, Mahil DS, Patel BP, Austen BM. Oligomerization and toxicity of β-amyloid-42 implicated in Alzheimer's disease. Biochem Biophys Res Commun. 2000; 273(3): 1003-7. doi: 10.1006/bbrc.2000.3051, PMID 10891362.
21. Patel R, Kaur K, Singh S. Protective effect of andrographolide against STZ induced Alzheimer's disease in experimental rats: possible neuromodulation and Aβ(1-42) analysis. Inflammopharmacology. 2021;29(4):1157-68. doi: 10.1007/s10787-021-00843-6, PMID 34235591.
22. Mushtaq N, Schmatz R, Pereira LB, Ahmad M, Stefanello N, Vieira JM, *et al.* Rosmarinic acid prevents lipid peroxidation and increase in acetylcholinesterase activity in brain of streptozotocin-induced diabetic rats. Cell Biochem Funct. 2014;32(3):287-93. doi: 10.1002/cbf.3014, PMID 24301255.
23. Schmatz R, Mazzanti CM, Spanevello R, Stefanello N, Gutierres J, Corrêa M, *et al.* Resveratrol prevents memory deficits and the increase in acetylcholinesterase activity in streptozotocin-induced diabetic rats. Eur J Pharmacol. 2009;610(1-3):42-8. doi: 10.1016/j.ejphar.2009.03.032, PMID 19303406.
24. Bhutada P, Mundhada Y, Bansod K, Tawari S, Patil S, Dixit P, *et al.* Protection of cholinergic and antioxidant system contributes to the effect of berberine ameliorating memory dysfunction in rat model of streptozotocin-induced diabetes. Behav Brain Res. 2011; 220(1): 30-41. doi: 10.1016/j.bbr.2011.01.022, PMID 21262264.
25. Baluchnejadmojarad T, Mansouri M, Ghalami J, Mokhtari Z, Roghani M. Sesamin imparts neuroprotection against intrastriatal 6-hydroxydopamine toxicity by inhibition of astroglial activation, apoptosis, and oxidative stress. Biomed Pharmacother. 2017; 88: 754-61. doi: 10.1016/j.biopha.2017.01.123, PMID 28157651.
26. Ionita R, Postu PA, Beppe GJ, Mihasan M, Petre BA, Hancianu M, *et al.* Cognitive-enhancing and antioxidant activities of the aqueous extract from *Markhamia tomentosa* (Benth.) K. Schum. stem bark in a rat model of scopolamine. Behav Brain Funct. 2017; 13(1): 5. doi: 10.1186/s12993-017-0123-6, PMID 28351401.
27. Cutler RG, Kelly J, Storie K, Pedersen WA, Tammara A, Hatanpaa K, *et al.* Involvement of oxidative stress-induced abnormalities in ceramide and cholesterol metabolism in brain aging and Alzheimer's disease. Proc Natl Acad Sci U S A. 2004; 101(7): 2070-5. doi: 10.1073/pnas.0305799101, PMID 14970312.

Cite this article: Shukla S, Sharma S, Vasudeva N, Hooda T. Danshensu Attenuates Diabetes Associated Cognitive Dysfunction by Markedly Reversing Oxidative Stress, Aβ and AChE Activity. Indian J of Pharmaceutical Education and Research. 2024;58(2):503-9.