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Original Article

Ameliorative Effect of Cichoric Acid against Diabetes Associated Cognitive Decline with Emphasis on Neurobehavioral Activity, Aβ and AChE

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ABSTRACT

Background: Diabetes Mellitus (DM) is closely related to degenerative and functional abnormalities of the CNS. Studies indicate that an alteration in the CNS brought on by persistent hyperglycemia is primarily responsible for diabetes-related cognitive impairment proving a clear connection between DM and cognitive impairment. Anti-oxidants, antihyperglycemics, and insulin-sensitizing substances can lessen this diabetes-related cognitive impairment. Due to the anti-diabetic and antioxidant characteristics, Cichoric Acid (CA) might have an impact on the cognitive impairment brought on by diabetes. **Aim:** The goal of the current investigation was to determine if CA protects against cognitive deterioration in Streptozotocin (STZ)-induced diabetic rats. **Materials and Methods:** Diabetes was induced by a single i.p. injection of Streptozotocin (STZ) 60 mg per kg of body weight. After development of persistent hyperglycemia rats were treated with Cichoric Acid for both acute (14 days) and chronic (28 days) conditions followed by behavioural and biochemical analysis. **Results:** The levels of $A\beta_{(1-42)}$, serum glucose, AChE activity, and MDA levels were markedly reduced after CA treatment in comparison to the STZ infused group. In contrast, the levels of GSH were significantly elevated by CA therapy. CA therapy improved learning and memory ability in both acquisition and probe trials (Morri's water maze tests), outperforming the STZ injection group. However, CA was found to mitigate the effects of STZ more significantly at the chronic level of treatment compared to the acute one. **Conclusion:** findings revealed that CA reduced cognitive impairment brought on by STZ, suggesting that CA has a lot of potential combat cognitive impairment.

Keywords: Diabetes mellitus, Cognitive dysfunction, Cichoric acid, Oxidative stress, Beta amyloid, Acetylcholinesterase.

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INTRODUCTION

The most prevalent endocrine illness, Diabetes Mellitus (DM), is defined by elevated blood glucose levels brought on by impaired insulin secretion, resistance to insulin action or both, impacting the eyes, kidneys, blood vessels, heart, and nerves.[1,2] Degenerative and functional abnormalities of the Central Nervous System (CNS) are closely related to DM.[3] A number of problems in neurochemistry, neurophysiology, and structural abnormalities are among the CNS impacts of diabetes.[4,5]

There is growing recognition that cognitive function diminishes in DM, which is frequently accompanied by severe consequences.[6-8] Reduced cognitive flexibility, psychomotor efficiency, and quick information processing are all observed in diabetic patients. Additionally, diabetic people appear to have a two-fold increased risk of Alzheimer's Disease (AD) and other dementias.[9] These facts indicate that DM and cognitive impairment are strongly related. Therefore, on the whole, metabolic abnormalities brought on by T2DM, including elevated glucose levels, peripheral hyperinsulinemia, altered lipid metabolism, and prolonged inflammation, trigger inadequate insulin delivery to the brain, resulting insulin resistance in neurons, which in turn causes increased synthesis and deposition of Amyloid β peptide (Aβ), ultimately leading neural degeneration and cognitive impairment.[10] However, like DM, Alzheimer's is the most prevalent type of disease among people and is defined by a progressive decline in memory and cognition ability. Aβ causes senile plaques, which in addition to neurofibrillary tangles made of hyperphosphorylated tau and, synapse loss, are the 3 main clinical hallmarks of AD from a neuropathological perspective. Aβ is linked to the generation of reactive nitrogen and oxygen species (RNS and ROS) and inducing calcium-dependent excitotoxicity,

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impaired cellular respiration, and disrupted synaptic processes that are involved in learning and memory.[11]

Moreover, diabetes is a chronic condition; ongoing metabolic stress and tissue damage continuously fuel aberrant free radical generation. Glucose auto-oxidation, decreased antioxidant defence enzyme synthesis and function, metabolic abnormalities brought on by high blood sugar levels, and mitochondrial destruction amidst the molecular processes contributes to oxidative stress in diabetes.[12]

It's interesting to note that some brain areas in diabetic model animals have higher levels of ROS, aberrant oxidative proteins, and higher levels of TBARS (thiobarbituric acid reactive species). On the other hand, oxidative radicals scavenging enzymes including SOD, CAT, or Glutathione (GSH) peroxidase frequently exhibit lower activity.[13] On the contrary side, the increased Aβ build-up in diabetic mice overexpressing APP (amyloid precursor protein) and deficient antioxidant enzymes indicate that enhanced oxidative damage has a significant effect on amyloid formation.[14] Thus, Oxidative stress is a key component of the shared pathogenesis of Type-2 DM and Alzheimer, and higher concentrations of ROS and RNS have repeatedly been observed in diabetic and Alzheimer patients. Additionally, it has been suggested that anti-oxidants, antihyperglycemics, and insulin-sensitizing substances can lessen diabetes-related cognitive impairment.[15,16]

Cichoric Acid (CA) is a hydroxycinnamic acid, an organic substance belonging to the phenylpropanoid class that is present in a wide range of plant species. It is both a caffeic and tartaric acid derivative.[17] First discovered in *Cichorium intybus*, CA also appears in substantial quantities in dandelion leaves, lemon balm, basil, aquatic plants, including algae and seagrasses, and *Echinacea*, particularly *E. purpurea*. According to reports, Cichoric acid possesses anti-cancer, anti-obesity, antiviral, and anti-diabetic characteristics.[18,19] Its anti- antioxidant potential is beneficial for many disorders.[20] Moreover, CA is reported to have a protective role in oxidative stress-induced cognitive loss.[21] Based on these data, we proposed that CA might have an impact on the cognitive impairment brought on by diabetes. However, no reports mentioning this problem. Therefore, the goal of the current investigation was to determine if CA protects against cognitive deterioration in Streptozotocin (STZ)-induced diabetic rats.

Therefore, the Morris water maze behavioral test and analysis of Aβ, AChE, MDA, and GSH levels in brain tissue of STZ-induced diabetic rats for acute as well as chronic situations were assessed to evaluate the neuroprotective role of CA. Moreover, the powerful antidiabetic drug Metformin is employed in this study to examine its antidiabetic effect in comparison to CA. Finally, Donepezil, a strong neuroprotective drug,[22] was used in this study as a benchmark to compare CA's impact on memory impairment.

MATERIALS AND METHODS

Animals

Wistar rats (180-220 g) were provided by Animal House at the Lala Lajpat Rai University of Veterinary and Animal Sciences in Hisar (Haryana, India). The animals were accommodated in a space with standard laboratory lighting, a humidity level, and free access to food and water. The animals received a one-week acclimation phase before the experimental work. Guidelines for all scientific activities involving animals were adopted as per 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 approved the study design during its meeting on February 11, 2019 (Endst. number of IAEC meeting minutes-IAEC/2019/10-19).

Chemicals

Before the experiment, all medication solutions were freshly made. The source of the Cichoric acid was Sigma Aldrich (Merk). Metformin and donepezil were bought from Cipla in India, and STZ was bought from SRL. Acetylthiocholine iodide was purchased from Sigma Aldrich (Merk). $A\beta_{(1-42)}$ was procured from Genxbio Health Sciences Pvt. Ltd.

In vivo experimental design

A total of 60 rats were obtained and fed flexibly for 7 days, 12 of which (the typical control group) received a basic diet. Streptozotocin (STZ) 60 mg/kg body weight was subsequently given as a single intraperitoneal injection to the remaining 48 rats.[23-25] Blood was collected from the tail vein, 72 hr after the injection, and blood glucose levels were assessed. Successful blood glucose levels in the diabetic models were those greater than 280 mg/dL. To test both acute and chronic therapy circumstances, these 48 diabetic rodents were randomly categorised into 2 sets of 4 groups (n=6 each), according to a predetermined protocol. The allocated groups are: Group 1-Control (Normal); Group 2-Diabetic-STZ group, which consists of diabetic rats not receiving any medication; Group 3-Metformin treated diabetic rats; Group 4-Donepezil (positive control) treated diabetic rats and Group 5-Cichoric acid treated group.

After giving these well-known diabetic rat models 4 weeks to develop persistent hyperglycemia, Cichoric acid was delivered intraperitoneally (i.p.) on day 39 at a dose of 1 mg/kg body weight for a period of 14 (acute) and 28 days (chronic). Here, we evaluate the impact of time and dose in treating diabetes associated AD using chronic and acute circumstances. In the diabetic rat models, metformin 100 mg per kg of body weight and donepezil 5 mg per kg of body weight (p.o.) were also given to the appropriate animal groups.

Morris water maze

In this case, the MWM test, which gauges learning as well as memory capacity, is used to gauge escape delay. The experiment was conducted in a 200 cm diameter circular pool with 25°C water temperature. The pool has four equally spaced quadrants. A platform with a diameter of 10 cm lies in the center of the quadrant, 2 cm below the water's surface. Over the course of four days, rats were instructed in each of the four quadrants for over 120 sec (once per day). It was observed how long it took to locate the platform (escape latency). The rat was led to the platform (and retained there for 20 sec) if it didn't reach there within 120 sec, at that point the latency was recorded as 120 sec. The platform was removed during the probing trial (day 5) and the rats had 120 sec to find it. Platform crossing frequency and the amount of time spent in the target quadrant were noted.[26,27]

Biochemical estimations

Blood glucose level

After receiving acute and chronic level treatments, blood was drawn from the tail vein to monitor blood glucose levels using a glucometer (Accu-check). Blood glucose levels was checked periodically during the experiment (10, 14, 20 and 28 days following the start of treatment). A glucose levels was measured in mg/dL.

Brain tissue preparation

The experimental investigation (acute and chronic) was followed by cervical dislocation in order to separate the brain tissues. The entire brain was placed in Phosphate-Buffered Saline (PBS) and homogenized in a glass Wise Stir Homogenizer after being carefully removed from the skull. The supernatant from the homogenized brain samples was then kept at -80°C for use in various biochemical experiments after being centrifuged at 10,000 g for 10 min at 4°C.

Estimation of protein

Based on the Biuret Method, the Erba Liquixx protein estimation kit was employed to calculate the amount of protein in the brain tissue of rat.

Measurement of Aβ

The beta-Amyloid (1-42) ELISA Kit (Genxbio Health Sciences Pvt. Ltd.,) was used to measure the amount of Aβ in line with the manufacturer's instructions.

Acetylcholinesterase (AChE) activity

Ellman *et al.*, (1961) spectrophotometric technique was applied to determine the AChE enzymatic activity with some modification.[28,29] The reaction mixture included 2.5 mL potassium phosphate buffer (pH 7.5), 0.1 mL homogenised brain material, 0.2 mL 5,5′-Dithiobisnitrobenzoic Acid (DTNB), and 0.2 mL acetylthiocholine iodide (75 mM). The optical density was determined at 412 nm following a 2 min incubation period at 25°C. For 2 min, optical density changes were monitored at 30 sec intervals. The amount of acetylthiocholine iodide hydrolyzed per minute per mg of protein was used to determine the AChE activity.

Estimation of Malondialdehyde (MDA)

A colorimetric method was used to gauge the amount of malondialdehyde present in the homogenate of brain tissue. A mixture of 0.5 mL of tissue homogenate and 0.5 mL of Tris HCl (pH 7.4) was maintained at 37°C for 2 hr. Following the addition of 1 mL of TCA (trichloro acetic acid), the mixture was centrifuged at 1000 g for nearly 10 min. Additionally, 1 mL of 0.67% thiobarbituric acid was added, and the mixture was allowed to sit in a boiling water bath for 1 hour. The thiobarbituric acid and MDA reaction, which results in the colour, was identified by a spectrophotometer at 532 nm.[30] The data were expressed in nmoles/mg protein.

Estimation of Reduced Glutathione (GSH)

The Reduced Glutathione levels were measured in the brain tissue homogenate using the Eillman method.[31] A mixture of 0.5 mL of sample and 0.5 mL of sulfosalicylic acid (4%), which stood for an hour at 4°C, was used. After that, the sample was centrifuged at 1200 g for roughly 5 min at 4°C. Then, 0.2 mL of 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB) and 2.7 mL of phosphate buffer were added to 0.5 mL of centrifuged supernatant (0.1M, pH 8). A spectrophotometer was used to measure the absorbance at 412 nm, and the outcomes were presented as nmoles of GSH per mg protein.

Statistical analysis

This experiment uses GraphPad Prism (version 5.0, Graph Pad Software, San Diego, CA). The data were statistically analysed using one-way ANOVA and repeated measure two-way ANOVA followed by Tukey *post hoc* test. Data were presented as mean±SEM. The p value less than 0.05 were deemed significant.

RESULTS

Morris water maze

Using the Morris Water Maze test (MWM), cognitive function was evaluated. In this behavioural experiment, after 14 and 28 days of therapy, animals underwent four days of training to locate a hidden platform. No significant differences on the first day of training in the Morris water maze among all 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. However, animal groups exposed to CA (for both acute and chronic level) showed a striking enhancement

in their learning ability throughout acquisition trials when compared to the STZ-infused group. According to the findings, learning considerably enhanced with CA administration in both acute and chronic treatment, and was equally significant ($p<0.001$) contrasted to the STZ-infused group as shown in Figure 1 (A). A comparative memory probe experiment was also carried out, and the outcomes revealed that the STZ-infused group had a substantially lower percentage of dwell time *vis-à-vis* normal, Metformin, and Donepezil-treated groups both over the 14 and 28 days of the investigation. In contrast to the STZ group, it is revealed from Figure 1(B) that animal groups exposed with CA showed a significant increase in % dwell time after 14 days ($p<0.05$) and 28 days ($p<0.01$) of therapy. Furthermore, compared to the control group, the STZ group displayed a significant drop in Platform Crossing Frequency (PCF). However, as shown in Figure 1 (C), the results clearly reveal that the animal group administered with CA considerably enhanced the platform crossing frequency relative to the STZ-infused group for acute ($p<0.05$) as well as chronic ($p<0.01$) treatment. Thus, it is clear from the above data that CA treatment for 28 days was slightly more effective than treatment for 14 days at mitigating the effects of STZ.

Blood glucose level

During both the acute and chronic phases of investigation, the STZ-infused rats showed upregulation ($p<0.001$) in the glucose level in contrast to the control group, demonstrating that the higher glucose level in rats causes cognitive impairment. One-way ANOVA demonstrate that both acute ($p<0.001$) as well as chronic ($p<0.001$) treatment with CA (1 mg/kg) and metformin ($p<0.001$) considerably decreased blood glucose when compared to STZ infused diabetic rats. Additionally, it is also clear from the data that glucose levels decreased significantly in the CA-treated group ($p<0.001$) in comparison to the Donepezil-treated group at both the acute and chronic levels of the study (Figure 2).

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

One-way ANOVA demonstrates that in comparison to the animals in the control group, the amount of $A\beta_{(1-42)}$ increased considerably following STZ administration ($p<0.001$). However, findings indicated that acute and chronic level treatment with CA showed significant ($p<0.001$) reduction in $A\beta_{(1-42)}$ levels as compared to the STZ group. Moreover, results also demonstrate that both acute and chronic level treatment, are equally significant in reducing the effects of STZ as when compared to the control group as seen in Figure 3.

Acetylcholinesterase (AChE) activity

Figure 4 shows the modifications in AChE activity following CA administration in the brain of rats. As seen, AChE activity was considerably higher in the brain of diabetes control or STZ administered rats ($p<0.001$) in comparison to the normal group. In contrary to the STZ group, rats administered with CA for 14 days showed an inhibition ($p<0.05$) in AChE enzyme activity. However, as shown in Figure 4, the effect of a 28-day CA therapy was more significant ($p<0.01$) in reducing the impact of STZ. This demonstrates that 28 days of CA therapy is more effective at reducing the activity of the AChE enzyme. Similar to this, donepezil therapy dramatically reduced the AChE activity in the rats' brain in comparison to the STZ infused rats.

MDA level

MDA serves as a marker for oxidative damage of tissue. Figure 5 shows the effects of acute as well as chronic CA therapy on Lipid Peroxidation (LPO). There was a substantial increase in levels of MDA in STZ infused group ($p<0.05$) as compared to the non-diabetic group. However, the rats group treated with CA considerably ($p<0.001$) decreased Malondialdehyde levels for both 14 and 28 days of the research, compared to the STZ-infused group. Additionally, post hoc analysis showed donepezil, and metformin significantly decreased MDA levels when compared to STZ infused group ($p<0.001$).

GSH level

Figure 6 shows the effects of acute as well as chronic CA therapy on brain GSH levels. In STZ infused rats ($p<0.001$) *vis-a-vis* non-diabetic control rats, there was a substantial decrease in the levels of GSH in the tissue of the rat brain. However, the rats group treated with CA considerably upregulated GSH levels for both 14 and 28 days of the research, compared to the STZ group. Moreover, results demonstrate that chronic level therapy ($p<0.01$) was more effective in attenuating effects of STZ than acute level therapy ($p<0.05$).

DISCUSSION

Studies suggest that DM is closely related to degenerative and functional abnormalities of the CNS.[32] There is growing recognition that cognitive performance diminishes in DM, which is frequently accompanied by serious problems. A growing number of studies indicate that alterations in the CNS brought on by persistent hyperglycemia are primarily responsible for diabetes-related cognitive impairment. These data point to a clear connection between DM and cognitive impairment.[33]

A rat model of diabetes using streptozotocin is widely known. This study looked at how the isoflavone CA affected the behaviour and biochemistry of STZ infused rat. Cognitive function significantly decreased as a result of Streptozotocin induced diabetes, associated with an increase in Aβ-peptide, AChE activity, and oxidative stress in the rats' brain. Acute and chronic CA therapy significantly reduced cognitive abnormalities, cholinergic impairment, and oxidative stress indicators in diseased rats. Additionally, CA therapy significantly corrected the behavioural and physiological changes brought on by STZ.[34]

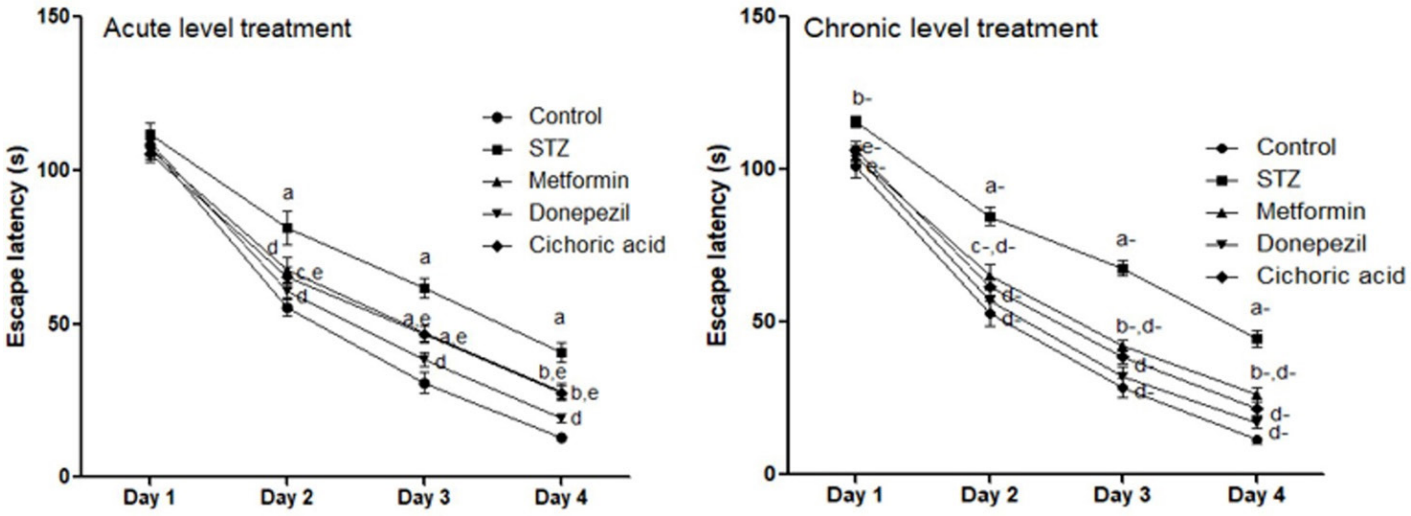

Figure 1 (A)

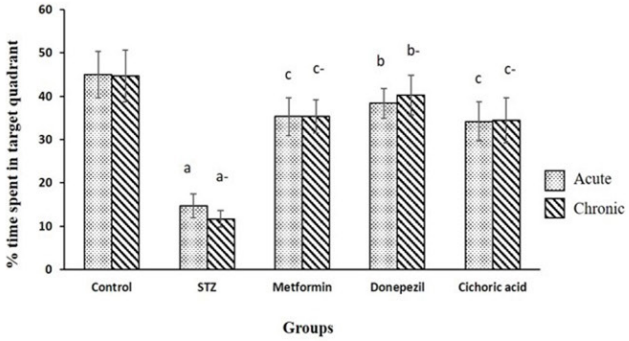

Figure 1 (B)

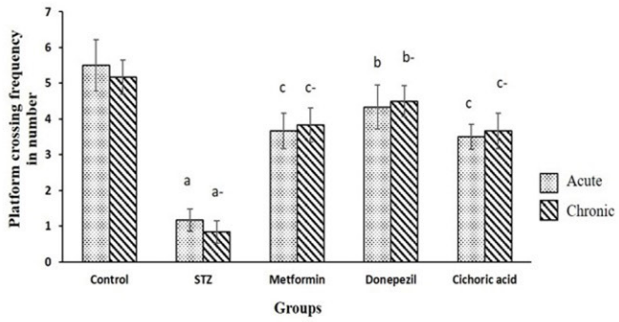

Figure 1 (C)

Figure 1: (A): Acute and chronic effects of CA on escape latency in the target quadrant of the Morris water maze. Two-way repeated measure analysis of variance and the Tukey-Kramer Multiple Comparison test were used to statistically analyze the data. The mean SEM is used to express the time values spent in the target quadrant. For acute level study results are a, (p<0.001) vs Control or Normal; b, (p<0.01) vs Normal; c, (p<0.05) vs Normal; d, (p<0.001) as compared to STZ; e, (p<0.01) as compared to STZ; whereas for chronic level study results are a-, (p<0.001) vs Normal; b-, (p<0.01) vs Normal; c-, (p<0.05) vs Normal; d-, (p<0.001) as compared to STZ; e-, (p<0.05) as compared to STZ. (B): Effect of CA treatment at both acute and chronic levels on the % time spent in the target quadrant in the Morris water labyrinth. The figure shows the effect 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. Each bar represents mean±SEM. For acute level study results are a, (p<0.001) in comparison to Control or Normal; b, (p<0.01) as compared to STZ; 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 to STZ; c-, (p<0.01) vs STZ (CA, Cichoric acid; STZ, Streptozotocin). (C): Effect of CA on platform crossing frequency in Morris water maze at acute as well as chronic levels. This figure illustrates the effect 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. The time devoted in the target quadrant is indicated by mean±SEM. For acute level study results are a, (p<0.001) compared to Control; b, (p<0.01) vs STZ; c, (p<0.05) as compared to STZ whereas for chronic level study results are a-, (p<0.001) as compared to Control; b-, (p<0.001) vs STZ; c-, (p<0.01) vs STZ (CA, Cichoric acid; STZ, Streptozotocin).

The memory and learning abilities of rats were examined here using the Morris water maze paradigm. As previously reported,[35] the MWM results demonstrated that STZ infused rats performed significantly worse than the non-diabetic rats as evidenced from a decline in time spent and PCF in the target quadrant during the probe phase, and a increment in escape latency throughout the acquisition trial. However, CA therapy improved learning and memory ability throughout the acquisition and probe trials, outperforming the STZ injection group. Moreover, the results of the current study show that diabetic rats' reduced performance is attributable to cognitive impairment rather than sensory abnormalities, as evidenced,[36] by the fact that diabetic rats' performance was comparable to that of normal rats in the experiment using a visible platform.

Prolonged hyperglycemia is said to be the main cause of most diabetes problems, including cognitive deficits.[37] and antihyperglycemic drugs and insulin sensitising medications are said to improve diabetic patients' memory.[38] In the current trial, CA administration dramatically decreased blood glucose levels, which is consistent with past investigations.[39] Therefore, the improvement in cognitive abilities seen in diabetic models in

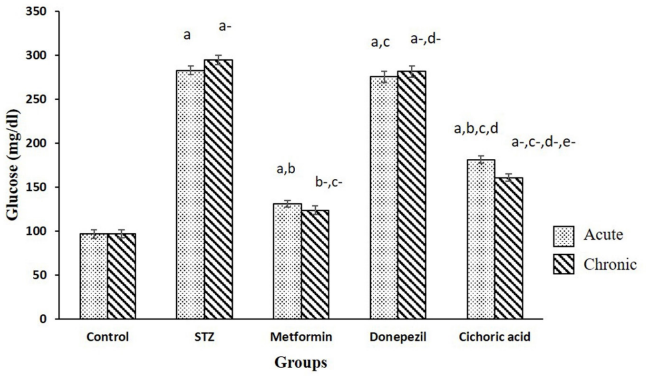

Figure 2: Effect of STZ on blood glucose level at acute as well as chronic level treatment. The graph depicts the impact of CA administration on blood sugar levels in diabetic and control rats at the start and end of the experiment. The values are depicted as mean±SEM. For acute level study results are a, (p<0.001) vs Control; b, (p<0.001) vs STZ; c, (p<0.001) vs Metformin and d, (p<0.001) vs Donepezil whereas for chronic level study results are a-, (p<0.001) vs Control; b-, (p<0.01) as compared to Normal; c-, (p<0.001) vs STZ and d-, (p<0.001) vs Metformin; e-, (p<0.001) in comparison to Donepezil (CA, Cichoric acid; STZ, Streptozotocin).

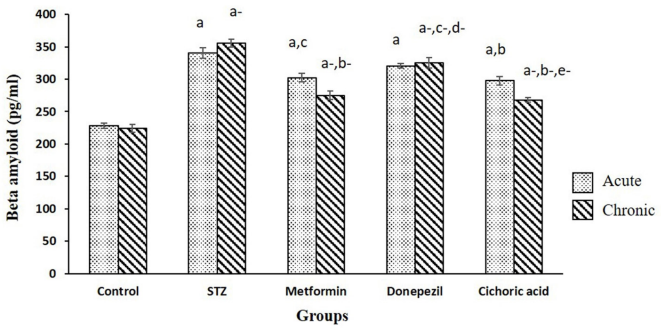

Figure 3: $A\beta_{(1-42)}$ concentrations in the brain of rats with diabetes induced by STZ and those receiving CA treatment. The figure depicts the effect of CA on $A\beta_{(1-42)}$ level activity of brain tissue in STZ administered rats. The values are depicted as mean±SEM. For acute level study results are a, (p<0.001) compared to Control; b, (p<0.001) vs STZ; c, (p<0.01) as compared to STZ; whereas for chronic level study results are a-, (p<0.001) in comparison to Control; b-, (p<0.001) vs STZ; c-, (p<0.05) compared to STZ; d-, (p<0.001) as compared to Metformin; e-, (p<0.001) vs Donepezil (CA, Cichoric acid; STZ, Streptozotocin).

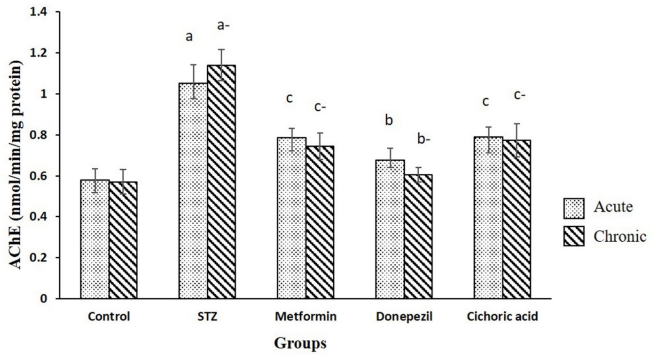

Figure 4: AChE activity in the brain of rats with diabetes induced by STZ and those receiving CA treatment. This figure illustrates the effect of CA on AChE activity of brain tissue in STZ administered rats. The values are depicted as mean±SEM. For acute level study results are a, (p<0.001) compared to Control; b, (p<0.01) 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) as compared to STZ; c-, (p<0.01) as compared to STZ (CA, Cichoric acid; STZ, Streptozotocin).

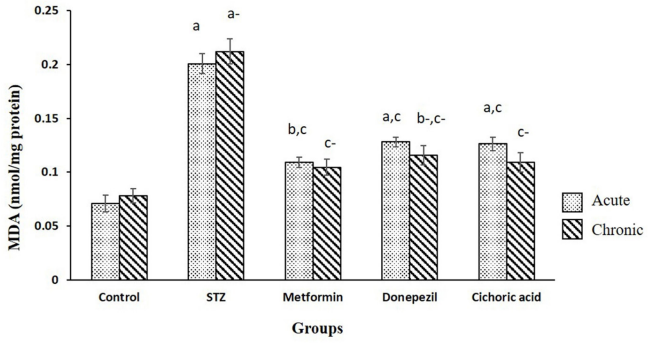

Figure 5: MDA levels in the brain of STZ-induced diabetic rats and those treated with CA. This figure demonstrates the effect of CA on MDA level of brain tissue in STZ administered rats. The values are depicted as mean±SEM. For acute level study results are a, (p<0.001) vs Control; b, (p<0.01) vs Control; c, (p<0.001) in comparison to STZ; whereas for chronic level study results are a-, (p<0.001) vs Control; b-, (p<0.05) vs Control; c-, (p<0.001) vs STZ (CA, Cichoric acid; STZ, Streptozotocin).

the current investigation after CA administration could be due to the anti-hyperglycaemic effect of CA. The current study showed that diabetic rats performed better in the MWM test after taking metformin, a well-known antihyperglycemic treatment, further supports this.[40]

As AD's distinctive neuropathological characteristic is the abnormal development of neuritic plaques and increased Amyloid Precursor Protein (APP) expression and Aβ deposition, which is seen in DM, are significant contributors to the formation of senile plaques in brain tissue.[41] In this study, an ELISA kit was used to measure the concentration of $A\beta_{(1-42)}$ in rats' brains. In both acute and chronic experiments, $A\beta_{(1-42)}$ levels in brain tissue were considerably greater in the STZ-infused animal group *vis-à-vis* control group.[42] However, treatment with CA resulted in the downregulation of $A\beta_{(1-42)}$ levels in comparison to the diabetic group. Moreover, results showed that both acute and chronic level treatment, are equally significant in reducing the effects of STZ when compared to the diabetic group. This downregulation of $A\beta_{(1-42)}$ level may be attributed to the antioxidant potential of CA.

A key mechanism supporting memory and cognitive function is cholinergic neurotransmission. However, numerous investigations have discovered that elevated AChE activity in the brain is linked to cognitive dysfunction in diabetes.[43] The current study's observation reveals that donepezil an AChE inhibitor,[44] improved diabetes-induced cognitive dysfunction provides additional evidence for this theory. Also, diabetes control or STZ-treated rats had significantly higher levels of AChE activity in their brains than the normal group.[45] Rats given CA for 14 days

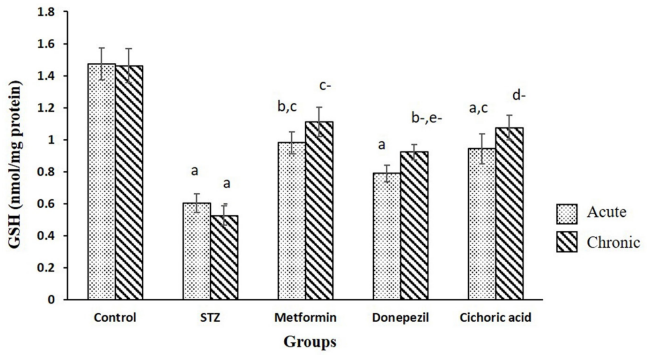

Figure 6: GSH levels in the brain of rats with diabetes induced by STZ and those receiving CA treatment. The figure demonstrates the effect of CA on GSH levels of brain tissue in STZ administered rats. The values are depicted as mean±SEM. For acute level study results are a, (p<0.001) vs Control; b, (p<0.01) compared to Control; c, (p<0.05) vs *STZ;* whereas for chronic 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) as compared to STZ*;* e-, (p<0.05) vs STZ (CA, Cichoric acid; STZ, Streptozotocin).

demonstrated suppression in AChE enzyme activity in contrast to the STZ group. However, a 28-day CA therapy had a more substantial influence on lowering the effects of STZ. This suggests that the AChE enzyme activity can be decreased more effectively using a 28-day CA treatment regimen.

A major contributing factor to neuronal injury appears to be an increase in reactive species generation brought on by oxidative stress. The cause of neuronal glucotoxicity is increased intracellular glucose oxidation. It has previously been suggested that oxidative stress to rat synapses in the hippocampus and cerebral cortex causes cognitive loss. Additionally, antioxidants are said to guard against diabetes-related cognitive impairment. After STZ treatment in the current study, lipid peroxidation levels considerably rose and GSH levels were significantly suppressed. CA, known for its antioxidant capabilities, significantly counteracted the negative effects of STZ on brain MDA and GSH levels.[45] Additionally, the reversal of STZ effects was more notable with chronic level therapy. These results indicate that CA provides a protective mechanism against oxidative stress (lower MDA levels and enhanced GSH levels), and it also plays a significant role in the reversal of memory and learning abilities.

CONCLUSION

Current study's findings suggest that the anti-diabetic, antioxidant, and AChE-inhibiting activity of CA may be the cause of its positive effects on STZ-induced memory impairment. The % time spent in MWM shows significant increase for Donepezil and Metformin treatment also so, supplementation CA in combination with Donepezil and Metformin may be beneficial. Additionally, it was found that CA treats STZ symptoms better at the chronic level than at the acute level. Therefore, CA could be practically applied to alleviate the cognitive and neurological impairment caused by diabetes.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

AD: Alzheimer's disease; **T2DM:** Type 2 diabetes mellitus; **Aβ:** Amyloid β peptide; **APP:** Amyloid precursor protein; **AChE:** Acetylcholinesterase; **ANOVA:** Analysis of variance; **MWM:** Morris water maze; **STZ:** Streptozotocin; **CAT:** Catalase; **CA:** Cichoric acid; **CNS:** Central nervous system; **LPO:** Lipid peroxidation; **DM:** Diabetes mellitus; **DTNB:** 5,5′-dithiobisnitrobenzoic acid; **ELISA:** Enzyme linked immunosorbent assay; **GSH:** Glutathione; **i.p.:** Intraperitoneally; **MDA:** Malondialdehyde; **p.o.:** Perose; **PBS:** Phosphate-buffered saline; **PCF:** Platform crossing frequency; **ROS:** Reactive oxygen species; **RNS:** Reactive nitrogen species; **SOD:** Superoxide dismutase; **TCA:** Trichloro acetic acid**; TBARS:** Thiobarbituric acid reactive species.

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