INVESTIGATION OF GLIMEPIRIDE IN STREPTOZOTOCIN AND SCOPOLAMINE INDUCED LEARNING AND MEMORY IMPAIRMENT IN RATS
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Abstract:
The present study was designed to evaluate the therapeutic potential of Glimepiride in Streptozotocin and Scopolamine induced Learning and Memory Impairment in Rats. Memory enhancing activity of glimepiride in STZ and scopolamine induced learning and memory impairment in rats was investigated by using Morris water maze. SOD, lipid peroxidation, glutathione level of brain homogenate was performed. Rats were randomly assigned to 9 groups (n = 6 each group): (1-5 Groups): control, STZ (60 mg/kg, i.p.), insulin (0.5 Units insulin per 100gm body weight), Glimepiride (5 and 10 mg/kg, p.o.) for 28 days. Again, (6-9 groups): Scopolamine (0.4 mg/kg, i.p) was administered 30 min before the evaluation of memory impairment for 5 days daily), Rivastigmine (2mg/kg, p.o.) and Glimepiride (1 and 2 mg/kg, p.o.) for 21 days. Administration of Glimepiride significantly restored learning and memory impairment induced by STZ and scopolamine in the Morris water maze, increased activity of brain antioxidant enzyme such as SOD, glutathione and also reduced the increased activity of lipid peroxidation. The Results concluded that glimepiride has improving effect on learning and memory impairment produced by STZ and scopolamine and it may have beneficial effect in the treatment of dementia.


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INTRODUCTION:
Learning is defined as the changes in behaviour, resulting from experience or mechanistically as changes in the organism that result from experience [1]. Long term change in learning is mental representation (mental imagery of things that are latest or old seen or sensed by sensory organ) and enhance due to experience. [2]
Simple non-Associative learning deal with the changes in behaviours that is due to symmetries in the presence of one stimulus. Such instances of learning are known as non-associative learning and Associative Learning: It is a process by which an association between two stimuli or behaviour and a stimulus is learned.
Memory is the condition for the behavioural change to be permanent and the natural counterpart of learning. It is the process of encoding, stored and retrieving of information. Encoding allows that information which is from external environment to reach our senses in chemical and physical stimuli form. Changing the information is the first stage, so that we may put the memory into the encoding system. Storage is the secondary stage of memory maintaining information over periods of time. Finally, the third process includes the retrieval of information from the stored one [3].
Disorders of learning and memory are like Alzheimer’s disease (AD) is a neurodegenerative disease described by progressive memory impairment, cognitive deficits and behavioural changes and is the most common form of dementia [4,5] followed by vascular dementia and dementia with Lewy bodies[6]. The disease has a multifactorial pathology characterised by aggregation of amyloid proteins [7], cholinergic deficits, oxidative stress, neuro inflammation[8,10]. Different forms of AD are with delirium, with delusions, with depressed mood and uncomplicated, each indicating the predominant feature of clinical presentation [9]. Amnesia is when someone sustains brain damage that results in a memory deficit. From bilateral damage the result of memory loss of different parts of the brain is significant for memory storage, processing, or recall. Dementia is a syndrome of weakening memory and other intellectual functions with little or no disturbance in consciousness. Degeneration of the cerebral neurons is one of the mutual and vital causes for dementia with increasing age [11].
Linkage between Acute Hyperglycaemia and Cognitive Impairment is that the end organ damage caused by Hyperglycaemia through increases in reactive oxygen species, in particular superoxide, which could lead to increase in activation of polyl pathway activation, increased formation of AGEs, activation of protein kinase C and increased glucose shunting in the hexosamine pathway. [12] Hyperglycaemia is the mark of all types of diabetes and could cause cognitive decrements by several different mechanisms. Acute changes in blood glucose are recognized to alter regional cerebral blood flow and could also cause osmotic changes in cerebral neurons. These same mechanisms may be operative in the brain induce the changes in cognitive function that have been detected in patients with diabetes. [13]
Mechanism Linking Cognitive Impairment and Diabetes Mellitus is like in patient with diabetes the development of cognitive dysfunction includes many hypotheses like potential causative role for hyperglycaemia, vascular disease, insulin resistance, hypoglycaemia and amyloid deposition. [14]
The Insulin and cholinergic hypothesis that suggests AD is caused by an inadequate production of acetylcholine may also have links to blood sugar abnormalities and insulin resistance. The researchers at Brown point out that insulin also participates significantly in neurological function by stimulating the expression of choline acetyltransferase (ChAT), the enzyme responsible for acetylcholine synthesis. Therefore suboptimal insulin levels as well as poor insulin receptor sensitivity can ultimately contribute to a decrease in acetylcholine, which further elucidates a possible biochemical link between diabetes and AD. [15] Second Generation, Glimepiride are more potent and probably safer than the first-generation [16]. These drugs are the second leading choice in anti-hyperglycaemic agents worldwide [17]. Although treatment aims essentially at lowering blood glucose and to allow for insulin release at a lower glucose threshold than normal, these drugs also appear to increase the risk of hypoglycaemia [16, 17]. Another highly relevant issue in second generation therapy is weight gain, which is important in relation between diabetes and obesity [18].
AD is characterized by both low insulin levels and insulin resistance within the central nervous system, as opposed to type 2 diabetes, which is characterized by high insulin levels and insulin resistance outside of the CNS. Insulin resistance and hyperinsulinemia cause a reduction in brain insulin [19]. Insulin receptors are found in areas of the brain responsible for cognition. Insulin activates signalling pathways associated with learning and long-term memory [20]. Insulin helps to regulate processes such as neuronal survival, energy metabolism, and plasticity. These processes are required for learning and memory. Peripheral insulin resistance therefore affects cognition [21]. In addition to regulating blood sugar levels, insulin functions as a growth factor for all cells, including neurons in the brain. Thus, insulin resistance or lack of insulin, in addition to adversely affecting blood sugar levels, contributes to degenerative processes in the brain [22]. When insulin levels reach an exceedingly high level, the beta-amyloid
peptide, the hallmark of AD that accumulates in senile plaques, is modulated [21,23]. Exaggerated elevation of plasma insulin levels causes amyloid peptide levels in the cerebrospinal fluid to increase, resulting in memory insult [22]. Glimepiride protects neurons against Ab-induced synapse damage and by reducing synapse damage could delay the progression of cognitive decline in AD [24].

Glimepiride which binds to the sulfonlyurea receptor SUR1 present on the pancreatic cells membrane which in turns stimulates insulin secretion by closing potassium channel [25] and have other extra pancreatic effects like increasing glucose uptake, stimulating the release of GPI-anchored proteins and activating PPARγ [26]. It docks to PPARγ and exhibits PPARγ agonistic activity in cell-based transactivation assay [27] and enhances the interaction of PPARγ with cofactors and up regulates the expressions of PPARγ target genes, including aP2 and leptin [26]. PPARγ activation is demonstrated to decrease levels of Aβ and senile plaques which improves the cognitive function in AD patients [18]. Due to similarity in structure with BACE-1 inhibitor sulphonamide it is indicated that glimepiride possess BACE-1 inhibition properties and thus down regulates Aβ. The result appears more promising and highlights the future role of glimepiride in treatment of AD associated with diabetes in lieu of fact that hyperglycaemia enhances Aβ 40 production and glimepiride significantly lowers hyperglycaemia induced Aβ 40 production [29].

MATERIALS AND METHODS:

Animals
Albino wistar rats weighing 250-300 g, were procured from animal house facility of Shri Guru Ram Rai Institute of Technology and Science, Patel Nagar, Dehradun. Animals were housed in an air conditioned animal room at 23±2°C with 12/12h light and dark cycle, with free access to water and food. The care of laboratory animals and all the procedures on animals were performed in strict accordance with the CPCSEA, Ministry of forest and environment Government of India. The protocol was approved by the Institutional animal ethics committee (registration no.264/PO/ReBi/S/2002/CPCSEA) and experiment was carried out in accordance with the CPCSEA guidelines.

Experimental Design
Animals were divided into nine groups and each groups comprising of six animals.
Group 1: Control group: Normal saline (1ml/kg, i.p.) was administered. 
Group 2: Disease control: Streptozotocin (60 mg/kg,i.p.) was administered after 15 minute of NAD+ (235mg/kg i.p.) administration. 
Group 3: Active Control group: Insulin as a standard was administered (0.5 Units insulin per 100gm body weight). 
Group 4: Test group 1: Streptozotocin (60 mg/kg, i.p.) + Glimepiride (5mg/kg, p.o.)
Group 5: Test group 2: Streptozotocin (60 mg/kg, i.p.) + Glimepiride (10mg/kg, p.o.)
Group 6: Disease control group: Scopolamine was administered 30 minutes before evaluation of Learning and Memory daily for 5 days.
Group 7: Active control group: Rivastigmine was administered (2mg/kg, p.o.). 
Group 8: Test group 1: Scopolamine (0.4mg/kg, i.p) induced learning and memory in dose of Glimepiride (1mg/kg, p.o.).
Group 9: Test group 2: Scopolamine (0.4mg/kg, i.p) induced learning and memory in dose of Glimepiride (2mg/kg, p.o.).

Experimental Induction of Learning and Memory Impairment
A single dose of Streptozotocin (60mg/kg i.p) prepared in citrate buffer (pH 4.4.0.1M) was intraperitonially administered to overnight fasted animals to induce diabetes. NAD+ 235mg/kg was administered prior 15 minutes to STZ administration. The control rats received an equal volume of citrate buffer and were used along with diabetic animals. Serum glucose level was estimated on 0th, 7th, 14th and 28th and 75th day respectively, after STZ administration by enzymatic GOD-POD (glucose oxidase peroxidase) diagnostic kit. The rats having fasting plasma glucose levels more than 250mg/dL were selective for the study [30]
Scopolamine (0.4 mg/kg) was used for impairment of learning and Memory in the present study for induction of Dementia

Behavioural models for evaluation of learning and memory:-
Morris water maze- MWM comprises of a circular tank having dimension of 60 cm in diameter and 25 cm in height. Up to 20 cm in depth MWM was filled. Water was made opaque with the help of milk. At 28±1°C temperature was maintained. The circular tank was divided with the help of thread into four equal quadrants (Q1, Q2, Q3 and Q4). A hidden platform was placed 1 cm below the surface of water in any one quadrant (Q4 in present study) painted white with top surface (6 cm × 6 cm). Each and every rat was subjected to four consecutive time trials each day, gap is of 5 min between trials.[31] 
Data was collected on basis of two trials during an acquisition Trial 4 trial change (one from each
starting position) per session was given for four days. The ceiling times of each trial are 0 sec. As the animal climbed on platform once. Before the commencement of next trial the animals stay there for about 20s. The time which was taken to reach the hidden platform was recorded as escape latency (within 120 sec). If animal was unable to find the platform within 120 sec then the was allowed to keep there for 20 sec.

<table>
<thead>
<tr>
<th>Day 1</th>
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Retrieval Trial - On the 5th day the determination of retention memory was carried out. The time spent in Quadrant which is previously hidden is recorded. The time spent in the target quadrant was noted down and it indicates the degree of memory consolidation.[32]

Biochemical Parameters for the Evaluation of Learning and Memory

Estimation of Serum glucose level [33, 34] Serum glucose level was estimated by glucose oxidase/ peroxidase method using commercially available enzymatic glucose oxidase- peroxidase method. PRINCIPLE: Glucose is oxidized to gluconic acid and hydrogen peroxide in the presence of glucose oxidase. Hydrogen peroxide further reacts with phenol and 4- amino antipyrine by the catalytic action of peroxidase to form a red colored quinonamine dye complex. Intensity of the color formed is directly proportional to the amount of glucose present in sample and it is measured photo metrically at 505 nm (490-530).

Estimation of Lipid peroxidation [35] Lipid peroxidase levels was estimated by Thiobarbituric acid reaction method described by Ohkawa et al. PRINCIPLE: The estimation of malondialdehyde (MDA), a product of lipid peroxidation was done by this method. One molecule of malondialdehyde reacts with two molecules of thiobarbituric acid (TBA) under mildly acidic conditions to form a pink color chromogen, the intensity of whose was measured in spectrophotometer 535nm.

Estimation of Reduced glutathione [36] The Estimation of reduced glutathione was done by the method of Ellman GL.
PRINCIPLE: Glutathione is a major non protein thiol and endogenous antioxidant that counters balance of free radical mediated damage. It is involved in the protection of normal cell structure and function by maintaining the redox homeostatis, quenching of free radicals groups. 5,5 di thio 2-nitrobenzoic acid (DTNB), a disulphide compound gets easily attacked by tissue sulphydryl group and forms a yellow coloured anion the intensity of which is measured at spectrophotometer at 412nm.

Estimation of Superoxide Dismutase [36] The superoxide dismutase activity in supernatant was measured by the method of Mishra and Fridovich.
PRINCIPLE: Superoxide dismutase is involved in the antioxidant defence against ROS by lowering the steady state oxygen level. SOD scavengers the superoxide ions produced as cellular by-products. This enzyme is a major defence for aerobic cells combating the toxic effect of superoxide radicals. It has the ability to inhibit the auto oxidation of epinephrine to adenochrome at pH 10.2. This inhibition can be measured with spectrophotometer at 480 nm. One unit of SOD is defined as the amount of enzymes requires producing 50% inhibition of epinephrine auto-oxidation.

Statistical Analysis:- Graph Pad Prism 7 Software was used to carried out the statistical analysis. All values were presented as mean ± SEM. The statistical significance of difference between means was calculated by one way Analysis of Variance (ANOVA) followed by Tukey’s and Bonferroni’s multiple comparison test in one behavioural model and biochemical evaluations except for escape latency in Morris water maze. Difference level at P<0.05 was considered statistically significant.

RESULTS:
Effect of STZ on Escape latency Time (ELT) and Time Spent in Target Quadrant (TSTQ) using Morris Water Maze in STZ treated rats. Control group showed significant decrease (p<0.001) in day 4 ELT as compared to its day 1. In control group rats spent significantly (p<0.001) more time spent in the target quadrant (Q4) when it compared to the time spent in other quadrants (Q1, Q2, Q3). STZ group showed a significant (p<0.001) increase in ELT as compared to ELT of control group on the same day. STZ treated rats also showed significant (p<0.001) decrease in time spent in target quadrant (TSTQ) when it is compared to time spent in target quadrant Q4 of control group.

Effect of Glimepiride on Escape Latency Time (ELT) of STZ treated rats using Morris Water Maze. Insulin treated group significantly (p<0.001) improved the ELT where compared with STZ treated group. Glimepiride (10mg/kg p.o.) showed more significant result (p<0.001) as compared to STZ treated group.

Effect of Glimepiride on Time Spent in target Quadrant (TSTQ) in STZ treated rats using Morris Water Maze.
When we compared among the control, STZ, STZ+ Insulin, STZ+ Glim (5 and 10 mg/kg p.o.) during time spent in targeted quadrant there is a significance difference. Administration of insulin showed high significance at (p<0.001) when compared to STZ Treated group. Glimepiride (5 and 10 mg/kg p.o.) showed high significance at(p<0.001) when compared to STZ treated.

Biochemical parameter

Effect of STZ on Serum glucose level.
Serum glucose level was estimated on 0th, 7th, 14th, 28th and 75th days in all STZ treated groups and the result was found significant in comparison to control group. The diabetic rats were selected whose serum glucose level was found more than 250 mg/dl after administration of STZ.

Effect of Glimepiride on serum glucose level in STZ induced diabetic rat
Glimepiride in two different doses 5mg/kg, 10mg/kg, was administered to diabetic rat and it was observed that the Serum Glucose level reduced as estimated after 10th and 28th days and effect was found significant.

Effect of Glimepiride on level of lipid peroxidation in STZ treated Rats.
When STZ is compared with the control group the levels of MDA during lipid peroxidation in brain produced a significant difference (p<0.001). Administration of Glimepiride (5 and 10 mg/kg) significantly (p<0.001) reduce the lipid peroxidation level as compare to disease control. Standard treatment with insulin significantly (p<0.001) reduce the level of lipid peroxidation in brain.

Effect of Glimepiride on level of superoxide dismutase (SOD) in STZ treated Rats.
Administration of STZ showed a significant (p<0.001) decrease in SOD levels in comparison with control group. However administration of Glimepiride (10 mg/kg) significantly (p<0.001) increase the level of SOD in brain while Glimepiride at a dose of 5 mg/kg showed a less significant (p<0.05) effect. Insulin also significantly (p<0.001) maintain the level of SOD.

Effect of Glimepiride on level of Glutathione in STZ treated Rats.
Administration of STZ produce a significant (p<0.001) decrease in glutathione level in comparison with control group. However treatment with of Glimepiride significantly (p<0.001) increase the glutathione level in brain. In other side the Standard treatment with insulin significantly (p<0.001) increase the level of glutathione.

Fig 1: Effect of STZ on Escape Latency time during Acquisition Trial in Morris water Maze.
Fig 2: Effect of STZ on TSTQ during Retrieval Trial in Morris water Maze.

Fig 3: Escape Latency of Rat using Morris water maze for 4 successive days (in sec)

Fig 4: Effect of Glimepiride on Time spent in Target Quadrant (TSTQ) during Retrieval trial in STZ treated rats on Morris water.

### Fig 2: Effect of STZ on TSTQ during Retrieval Trial in Morris water Maze.

- **a =** \( p < 0.001 \) vs Time spent in other quadrant in STZ treated group.
- **b =** \( p \leq 0.001 \) vs time spent in target quadrant Q4 of STZ treated group.

### Fig 3: Escape Latency of Rat using Morris water maze for 4 successive days (in sec)

- **a =** \( p \leq 0.001 \) vs ELT on first day of STZ treated group
- **b =** \( p \leq 0.001 \) vs ELT on STZ treated group.

### Fig 4: Effect of Glimepiride on Time spent in Target Quadrant (TSTQ) during Retrieval trial in STZ treated rats on Morris water.

- **a =** \( p \leq 0.001 \) vs. time spent in other quadrants in STZ control group.
- **b =** \( p \leq 0.001 \) vs. time spent in target quadrant Q4 of STZ control group.
a= p≤0.001 as compared to Control and STZ group.

Fig 5: Effect of STZ on serum glucose level in Rats.

a=p≤0.01, versus Control group
b=p≤0.05, versus STZ group

Fig 6: Effect of glimepiride on Serum glucose level in rats

a= p≤0.0001 versus normal control.

b = p≤0.001 versus STZ group.

c = p≤0.001 versus STZ+ GLIM (5 mg/kg).

Fig 7: level of lipid peroxidation (nM/mg protein) on STZ treated rats.
DISCUSSION:
Alzheimer’s disease is slowly or gradually progressive dementia affecting both cognition process and behaviour [37]. It damage and kills brain cells. It comprises of alternations in Learning and memory. The pathological states of AD are senile plaques, Acetylcholine deficit, inflammation, neurofibrillary tangles, Oxidative stress and changes in the process of Neurotransmitters [38]. Learning is defined as the act of obtaining new or making partial changes to existing knowledge, behaviour and skill [39]. Memory is the process in which captured information is encoded, stored and retrieved. Learning and memory impairment is closely related to the cholinergic hypothesis [40]. Learning and memory impairment due to diabetes is associated with a pathological condition called Hyperglycaemia. It induced ROS, Neuronal degeneration and promotes neuronal damage. Oxidative stress is the main cause of memory impairment; it is the excessive production and/or insufficient removal of ROS. Oxidative stress affected Amyloid precursor protein (APP) by amending its processing and causing accumulation of Aβ this show that there is some alteration in the
level of ROS and RNS. Both of these pathways involved in DM induced memory impairment. As Hyperglycaemia increase the glucose level which leads to increase in the formation of AGE’s. On the other hand AGE’S increase the expression of RAGE’s this will lead to AGE’s and RAGE’S interactions which causes activation of neurofibrillar tangles. The deposition of Aβ causes neurodegenration and activation of NF-kB which causes neuroinflammation. Both neurodegeneration and neuroinflammation leads to Diabetic memory impairment [41]. For the evaluation of learning and memory one behavioural model was used in STZ treated rat’s i.e. Morris water maze. This present study shows the significant role that GLIM plays important role in enhancing learning and memory. This increase in time spent in Target quadrant and decline in escape latency on 5th day shows the memory enhancing activity of GLIM.

Present study evaluated that the oxidative stress which was induced by STZ in rats decreased the SOD and GSH and the treatment with GLIM increase the level of both the antioxidant enzymes and it may possibly decrease the formation of ROS and nerve cell death. The oxidative stress induced by STZ and it lead in increase amount of lipid peroxidation in the brain shows the neuronal damage. The administration of GLIM is effective in reducing the level of lipid peroxidation. The decrease of antioxidant defence and/or increase in free radical generation deteriorates the pro oxidant and antioxidant balance regulation which leads to oxidative stress and cell death.

In STZ induced rats, Hyperglycaemia induced the oxidative stress and it may lead to neuronal/damage and death. This damage is mainly caused by increase ROS formation, increase MDA level and decrease SOD and GSH level. This overall study shows the antioxidant property of GLIM significantly reduced the level of lipid peroxidation and increases the level of antioxidants (SOD, GSH).

Infact the administration of GLIM in our experiment was potentially active in reducing the oxidative stress. This indicates that GLIM has potent antioxidant activity to reduce the oxidative stress induced lipid peroxidation.

CONCLUSION:
The aim of the present study was designed to investigate the effect of Glimepiride in STZ and Scopolamine induced learning and memory impairment in rats. In this experiment AD was induced by a single dose (60 mg/kg) of STZ and by scopolamine (0.4 mg/kg) in rats. Induction of STZ causes hyperglycemia which leads to increase in the formation of ROS. ROS causes the Neuronal damage and hence learning and memory impairment in first half groups.

- In STZ treated group there is increase in the level of blood glucose which will lead to hyperglycemia induced impairment in learning and memory, Glimepiride show significant decrease in the blood glucose level of rats.

STZ and Scopolamine treated rats showed:
- Significant increase in the level of lipid peroxidation which was reversed by the treatment with Glimepiride which is indicated by decrease in the toxicant elevated levels of thiobarbituric acid reactive substances (TBARs) like MDA.
- The activity of antioxidants like SOD, GSH in STZ and Scopolamine treated group is decreased, the activity is restored after treated with Glimepiride by reducing the generation of free radicals.
- Decrease in Morris water maze in Glimepiride treated groups shows improvement in learning and memory.

Disease induced rats showed a significant increased activity is reverse by treatment of group with GLIM and standard. Hence, overall study concluded that Glimepiride have a potential role in the management of cognitive dysfunctions like learning and memory impairment.

REFERENCES: