"ATTENUATION OF COGNITIVE DYSFUNCTION BY TELMISARTAN IN COMBINATION WITH LUTEOLIN IN HIGH-FAT DIET-STZ-INDUCED DIABETES IN EXPERIMENTAL ANIMALS."

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Abstract

Objective: To study effect of telmisartan with luteolin on cognitive dysfunction in high fat diet-STZ induced diabetic rats.

Material and Methods: HFD-fed rats were then injected with STZ (30 mg/kg, i.p.) to induce diabetes and were subsequently fed a HFD for an additional nine weeks. Cognitive impairment was assessed using Morris water maze and elevated plus maze models. Other parameters such as glucose levels, body weights, GSH, as well as LOP, were estimated in brain homogenates. **Results:** The combination treatment groups of telmisartan with luteolin exhibited a prominent decrease in glucose levels and were able to restore body weight compared to the diabetic control group and other individually treated groups. TL-I and TL-II demonstrated remarkable significance in attenuating escape latency in the water maze and transfer latency in the elevated plus maze, respectively, compared to the diabetic control group and all individually treated groups. Telmisartan plus luteolin might be due to decrease in oxidative stress via antioxidant effect of luteolin which scavenges free radicals and restores normal levels of endogenous antioxidants as well as the effect of telmisartan to decrease the glucose toxicity induced oxidative stress and cellular damage responsible for attenuation of memory in rats.

Conclusion: Based on the present investigation, it can be concluded that the combination of telmisartan with luteolin synergistically attenuated cognitive dysfunction in Type 2 diabetic rats.

Keywords: Telmisartan, Morris water maze, Type 2 diabetes, Luteolin.

Introduction

The primary cause of dementia, specifically Alzheimer's disease (AD), is characterized by the accumulation of neurofibrillary tangles in the brain. An increasing body of research suggests that type 2 diabetes mellitus (T2DM) may have a significant role in the development of Alzheimer's, either directly or indirectly. Recent studies have shown that diabetes could potentially cause adverse effects on the central nervous system (Liu et al., 2013).

Multiple investigations have demonstrated that individuals with type 2 diabetes are at a substantial risk for the abnormalities observed in Alzheimer's disease. Furthermore, both conditions share similar molecular and physiological processes, such as hyperglycemia, insulin resistance, the formation of amyloid, and neuronal damage caused by free radicals. Insulin resistance, leading to the development and progression of Alzheimer's, is likely responsible for a range of pathogenic changes seen in dementia. Subsequent research studies have provided substantial evidence suggesting that diabetes may contribute to memory loss. Several risk factors associated with the development of memory loss among individuals with diabetes include insulin resistance, weight gain, chronic low-grade inflammation, and more (Jash et al., 20).

Type 2 diabetes mellitus (T2DM) is at risk due to obesity. In addition to causing metabolic problems and insulin resistance (IR), obesity also encourages beta-cell malfunction (Muriach et al., 2014). The most obvious T2DM sign is insulin resistance. Insulin resistance at the cellular level prevents proper utilization of glucose in the blood, resulting in hyperglycemia. This leads to increased oxidative damage in organs like the brain, triggering a chronic inflammatory response (Pugazhenthi et al., 2017).

In the model, a combination of a high-fat diet and streptozotocin treatment (HFD/STZ) in rats or mice is an example that mimics the human diet. It involves administering a meal rich in fat and, at times, sugar, along with the administration of the cell toxin streptozotocin. This combination induces insulin resistance, glucose intolerance, hyperglycemia, and results in a significant reduction in body weight. These two stressors are intentionally combined to replicate the pathophysiology of diabetes, particularly type 2 diabetes, albeit within a shorter timeframe compared to that observed in human diabetes.

Telmisartan, an angiotensin II blocker, is utilized for the treatment of hypotension. Additionally, it functions as a partial PPARγ agonist. Apart from regulating blood pressure, telmisartan also exhibits the ability to decrease oxidative stress and activate nuclear receptors involved in the metabolism of carbohydrates and lipids (Amal and Hala, 2007).

Flavonoids, known for their diverse biological effects, possess properties such as anti-inflammatory, neuroprotective, and antioxidant activities. Recent research suggests that dietary flavonoids, including luteolin, which has recently garnered significant interest, may have the potential to improve memory and neurocognitive function in humans (Liu et al., 2013). Both drugs demonstrate significant

potential in the treatment of diabetes and its complications individually. However, their combined effect on cognitive dysfunction associated with diabetes in experimental animals has not yet been assessed. Therefore, the present investigation was undertaken to determine whether the combination of these drugs produces a synergistic effect in attenuating cognitive dysfunction in Wistar rats.

MATERIAL AND METHODS

Drugs and chemicals

Telmisartan was obtained as gift sample from Ascend Laboratories, Mumbai, and Maharashtra, India. Streptozotocin and luteolin were procured from Sigma Aldrich, USA. High Fat Diet was purchased from Research Diet Inc., New Jersey, USA.

Experimental animals

Wistar rats weighing between 200-220g male were obtained from LACSMI, Biopharms PVT. LTD., Pune. The study protocol was approved by the Institutional Animal Ethics Committee of KBHSS trusts Institute of pharmacy, Malegaon (IAEC registration no. 1566/PO/Re/S/11/CPCSEA), and all experimental procedures were carried out in compliance with CPCSEA guidelines.

Experimental protocol

Eight-week-old male wistar rats were fed a HFD (Research Diet Inc., New Jersey, USA) for 4 weeks. HFD-fed rats were then injected with STZ (30 mg/kg, i.p.) to induce diabetes and then were fed with a HFD for nine more weeks (Correia-Santos et al., 2012).

Rats with blood glucose level higher than 250 mg/dl was be regarded as diabetic and involved in the study protocol. Forty-two rats were randomly divided into seven groups with six rats in each. The normal group contained rats fed with a normal diet i.e. food chow pellet (Nutrivet Life Sciences, Pune, India).

Total of 48 rats was be used out of which eight were nondiabetic (normal).

The rats in group I was be nondiabetic and rats in group II to group VII was be diabetic.

High Fat Diet and tap water ad libitum for 9 more weeks. All tests were conducted between 08:00 and 13:00 h. The rats were divided in to 8 groups (n=6) as follows

NC receive 1% CMC (1 ml/kg, p.o.), DC receive 1% CMC (1 ml/kg, p.o.), TEL- I receive telmisartan (5 mg/kg, p.o.), TEL- II receives telmisartan (10 mg/kg, p.o), LUT receive luteolin (10 mg/kg, p.o), TL – I receive telmisartan (5 mg/kg p.o.) plus luteolin (10 mg/kg p.o.), TL-II receive telmisartan (10 mg/kg p.o.) plus luteolin (10 mg/kg p.o.), PIO receive pioglitazone (30 mg/kg p.o.

Biochemical parameters from blood

At 1st and last day of experiment, all the animals were anesthetized with isoflurane (gas) anesthetic and blood was withdrawn by puncturing retro-orbital plexus by using fine glass capillary and collected in epindorff tubes. Serum was separated by centrifugation and was used for estimation of serum glucose (GOD/POD Method).

Morphological parameters

Body weight of rats was measured on day 1 and 90 days of treatment.

Morris Water Maze Model

Animals were tested for spatial memory assessment using Morris water maze test which was established by Morris et al., 1982. The test apparatus was circular water tank (180 cm in diameter and 60 cm high) made up of dark gray plastic that was partially filled with water (24 ± 1 C). Full cream milk (1.5 l) was used to render the water opaque. The pool was divided virtually into four equal quadrants, labeled W– X–Y–Z. A platform which has dimensions 12.5 cm in diameter and 38 cm height was placed in one of the four maze quadrants and submerged 2 cm below the water surface level.

a) Spatial memory assessment

It was done by using Morris water maze in last five days of experiment i.e. 86 to 90 days. The rat was placed facing the wall of the pool at the starting position of each quadrant. When the rat was positioned in the water, timing began immediately, and when the rat climbed (touched) the platform, the time was stopped and recorded. If the rat failed to locate the platform within the 120s, it was gently placed on platform and allowed to remain there for 30s. Once the test was completed, another animal was employed for the test in the same quadrant. After all animals had completed the test in the first quadrant, the tests were repeated in the second quadrant until all four quadrants were covered. The time to reach the platform (escape latency) was measured. After every trial rats were dried by using cotton cloths and then they were transferred to respective cages.

b) ***Probe trial test***

It was conducted on 91 day of treatment. After a 24-hour navigation test in a water maze, the target platform was removed, and the rat was put back in the maze facing the wall in the same position where the platform was originally located. Record the time it took for the rat to pass the original location of the platform. If it took more than 30 seconds, the rat was taken out of the maze. This was done to test the rat's memory of the platform's location.

Elevated plus-maze model

Retention of learning and memory can be assessed by using elevated plus maze (Itoh et al., 1990). The elevated plus-maze consisted of two opposite open arms, 50×10 cm, crossed with two enclosed arms, of the same dimensions with 40 cm high walls. The arms were connected with a central square (10×10 cm) to give the apparatus a plus sign appearance. The maze was elevated 50 cm above floor level. On day 1, a rat was individually placed on the end of one of the open arms, facing away from the center, and the time taken by the animal to enter one of the closed arms (transfer latency (TL) day 1 (i.e. 86 day) was recorded with the help of a stop watch. The rat was left in the enclosed arm for 10–15 s and returned to its home cage. The rats unable to enter closed arm within 90 s were placed in closed arm. On day 2, the procedure was repeated and the day 2 (i.e. 87 day) TL was recorded.

Study of biochemical parameters from brain

Brain was also removed and weighed in all group of animals. Brain homogenates were prepared in cold 50 mM Tris buffer (pH 7.4) using Remi homogenizer so that clear homogenate was formed. The supernatant of brain homogenate was used for the estimation

of GSH (Ellaman, 1959), malondialdehyde (MDA) (Slater & Sawyer, 1971) levels.

Result

Effect on escape latency

In the normal control group, the escape latency gradually decreased. In diabetic control (DC) group there was significant increase ($p<0.001$) in the escape latency on day 88, day 89 and day 90 when compared with normal control group. TEL-I groups showed significant decrease in escape latency on day 89 ($p<0.05$) and day 90 ($p<0.01$) when compared with DC group.

TEL-II group showed significant decrease in escape latency on day 89 ($p<0.01$) and day 90 ($p<0.001$) when compared with DC group. LUT groups showed significant decrease ($p<0.01$) in escape latency on day 89 and day 90 when compared with DC group. PIO, TL-I and TL-II groups showed significant decrease ($p<0.001$) in escape latency on day 89 and day 90 when compared with DC group. TL-I groups showed significant decrease in escape latency on day 89 when compared with TEL- I ($p<0.01$), TEL-II ($p<0.05$), LUT ($p<0.01$) groups.

TL-I groups showed significant decrease in escape latency on day 90 when compared with TEL- I ($p<0.001$), TEL-II ($p<0.01$), LUT ($p<0.001$) groups. TL-II groups showed significant decrease in escape latency on day 89 ($p<0.01$) and day 90 ($p<0.001$) when compared with TEL-II group (Table 1 and Figure 1).

Table 1: Effect of combination of telmisartan with luteolin on escape latency in Morris water maze in High-fat diet and streptozotocin-induced cognitive dysfunction in rats.

	Escape latency (sec) on				
Group	**DAY 86**	**DAY 87**	**DAY 88**	**DAY 89**	**DAY 90**
NC	113.20 ± 6.06	80.13 ± 8.33	63.25 ± 5.59	45.88 ± 4.12	26.63 ± 1.49
DC	114.25 ± 2.47	99.20 ± 6.76	88.80 ± 4.66$^{\#\#}$	72.60 ± 5.80$^{\#\#}$	68.34 ± 2.16$^{\#\#}$
PIO	104.33 ± 5.67	91.17 ± 5.84	60.33 ± 4.93	46.25 ± 5.56***	30.45 ± 1.73***
TEL-I	101.17 ± 4.76	89.04 ± 5.78	87.50 ± 3.85	63.54 ± 5.94*	57.56 ± 2.11**
TEL-II	102.78 ± 3.33	85.83 ± 4.16	88.75 ± 4.62	61.04 ± 4.89**	54.32 ± 2.17***
LUT	104.71 ± 2.93	92.71 ± 5.79	82.14 ± 4.27	63.15 ± 6.72*	56.80 ± 2.52**
TL-I	108.60 ± 4.40	76.33 ± 4.93	68.60 ± 5.60	52.54 ± 4.42^{***,aa,b,cc}	42.45 ± 1.84^{***aaabbccc}
TL-II	101.97 ± 3.65	79.17 ± 4.00	63.17 ± 5.81	47.68 ± 5.84***, ^{aaa,bb,ccc}	33.45 ± 2.01^{***aaa,bbb,ccc}

Na CMC- Sodium Carboxy methyl cellulose, NC- Normal control, DC- Dexamethasone control, PIO- Pioglitazone, TEL- Telmisartan, LUT- Luteolin, TL - Telmisartan plus Luteolin. Results are presented as mean ± SEM, (n=6). ANOVA followed by Dunnett test.

$^{\#\#\#}p$ <0.001 when compared with NC. $^{***}p<0.001$, $^{**}p<0.01$, $^{*}p<0.05$ when compared with DC. $^{aaa}p<0.001$, $^{aa}p<0.01$, $^{a}p<0.05$ when compared with TEL- I. $^{bbb}p<0.001$, $^{bb}p<0.01$, $^{b}p<0.05$ when compared with TEL-II, $^{ccc}p<0.001$, $^{cc}p<0.01$, $^{c}p<0.05$ when compared with LUT

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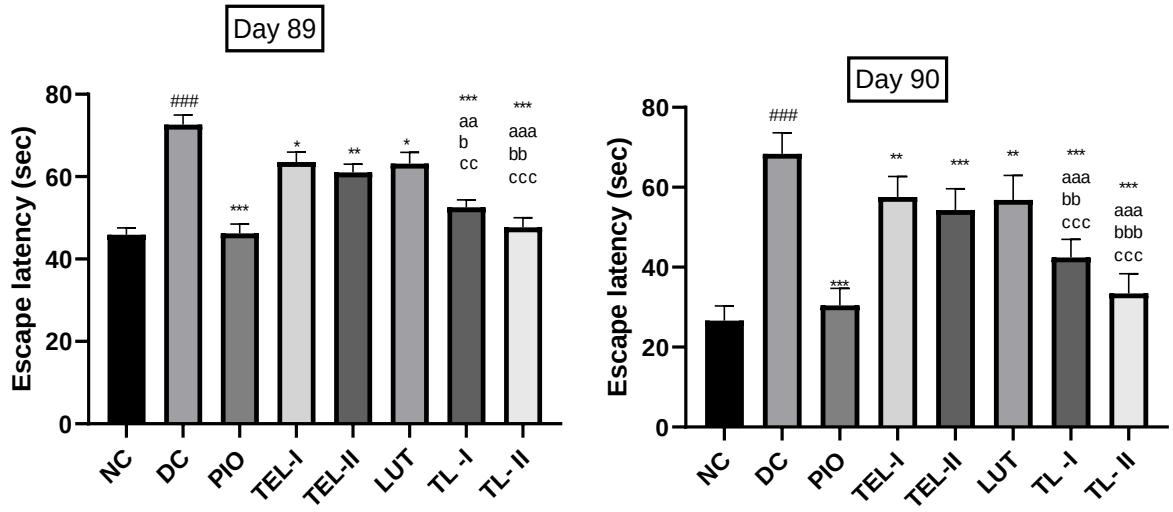
Figure 1: Effect of combination of telmisartan with luteolin on escape latency in Morris water maze in High-fat diet and streptozotocin-induced cognitive dysfunction in rats.



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Probe trial test (Time require to locate target quadrant)

In diabetic control (DC) group showed significantly more time require to reach target quadrant ($p<0.001$) on day 91 when compared with normal control group. TEL-I groups showed significantly less time require to reach target quadrant ($p<0.05$) on day 91 when compared with DC group. TEL-II group showed significantly less time require to reach target quadrant ($p<0.001$) on day 91 when compared with DC group.

LUT groups showed significantly less time require to reach target quadrant ($p<0.01$) on day 91 when compared with DC group. TL-I group showed significantly less time require to reach target quadrant ($p<0.001$) on day 91 when compared with DC group, TEL- I, TEL-II), LUT groups. TL-II groups showed significantly less time require to reach target quadrant ($p<0.001$) on day 91with DC, TEL- I, TEL-II), LUT groups (Table 2 and Figure 2).

Table 2: Effect of combination of telmisartan with luteolin probe trial test in high fat diet and streptozotocin-induced cognitive dysfunction in rats.

Group	Time spent (Sec)
	Target Quadrant
NC	06.56 ± 0.35
DC	$27.42 \pm 2.21^{###}$
PIO	$08.81 \pm 0.88^{***}$
TEL- I	$24.32 \pm 2.28^{*}$

TEL- II	$22.14 \pm 1.56^{***}$
LUT	$23.37 \pm 1.37^{**}$
TL- I	$15.56 \pm 1.40^{***, aaa,bbb,ccc}$
TL- II	$11.41 \pm 1.25^{***, aaa,bbb,ccc}$

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$^{###}p$ <0.001 when compared with NC. $^{***}p<0.001$, $^{**}p<0.01$, $^{*}p<0.05$ when compared with DC. $^{aaa}p<0.001$, $^{aa}p<0.01$, $^{a}p<0.05$ when compared with TEL- I. $^{bbb}p<0.001$, $^{bb}p<0.01$, $^{b}p<0.05$ when compared with TEL-II, $^{ccc}p<0.001$, $^{cc}p<0.01$, $^{c}p<0.05$ when compared with LUT

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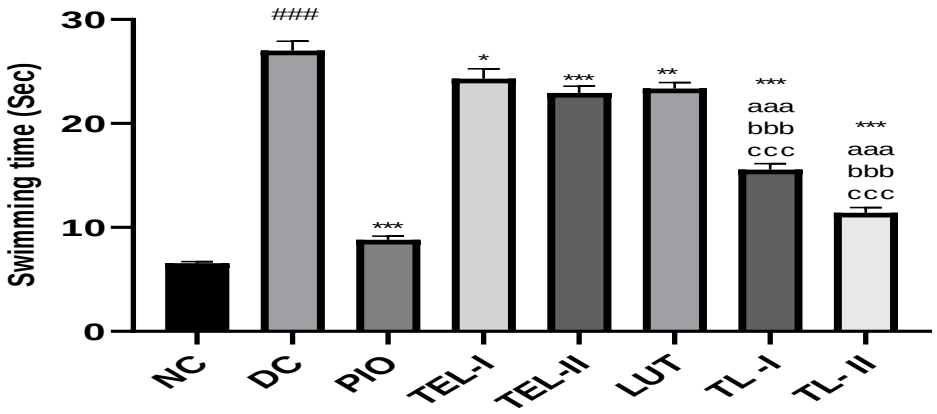
Figure 2: Effect of combination of telmisartan with luteolin probe trial test in high fat diet and streptozotocin-induced cognitive dysfunction in rats.



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$^{###}p$ <0.001 when compared with NC. $^{***}p<0.001$, $^{**}p<0.01$, $^{*}p<0.05$ when compared with DC. $^{aaa}p<0.001$, $^{aa}p<0.01$, $^{a}p<0.05$ when compared with TEL- I. $^{bbb}p<0.001$, $^{bb}p<0.01$, $^{b}p<0.05$ when compared with TEL-II, $^{ccc}p<0.001$, $^{cc}p<0.01$, $^{c}p<0.05$ when compared with LUT.

Transfer latency on elevated plus-maze

In the normal control group, the transfer latency gradually decreased. In diabetic control (DC) group there was significant increase ($p<0.001$) in the transfer latency on day 86, day 87 when compared with normal control (NC) group. PIO group showed significant decrease ($p<0.001$) in transfer latency day 86, day 87 when compared with DC group. TEL-I groups showed significant decrease in transfer latency on day 86, day 87 ($p<0.01$) when compared with DC group. TEL-II group showed significant decrease in transfer latency on day 86 ($p<0.001$) and day 87 ($p<0.001$) when compared with DC group. LUT groups showed significant decrease ($p<0.05$) in transfer latency on day 86 and day 87 when compared with DC group. TL-I & TL-II groups showed significant decrease ($p<0.001$) in transfer latency on day 86 and day 87 when compared with DC group. TL-I groups showed significant decrease in transfer latency day 86 when compared with TEL- I ($p<0.001$),

TEL-II (p<0.05), LUT (p<0.001) groups. TL-I groups showed significant decrease in transfer latency day 87 when compared with TEL- I (p<0.01), TEL-II (p<0.05), LUT (p<0.01) groups. TL-II groups showed significant (p<0.001) decrease in transfer latency day 86 and day 87 when compared with TEL- I, TEL-II, LUT groups (Table 3 and Figure 3).

Transfer latency (TL) on day 86 and day 87 of treatment		
Groups	**Day 86 TL(Sec)**	**Day 87 TL after 24hr (Sec)**
NC	40.75 ± 2.06	28.50 ± 2.72
DC	70.20 ± 2.98$^{\#\#}$	66.40 ± 2.41$^{\#\#}$
PIO	47.76 ± 3.15***	40.45 ± 2.56***
TL-I	64.20 ± 2.44**	52.80 ± 2.45**
TL-II	58.89 ± 2.20***	51.33 ± 2.88**
LUT	65.50 ± 2.63*	53.44 ± 4.39*
TL-I	54.55 ± 2.95^{***,aaa,b,ccc}	37.43 ± 2.32^{***,aa,b,cc}
TL-II	40.33 ± 2.32^{***,aaa,bbb,ccc}	31.50 ± 1.20^{***,aaa,bbb,ccc}

Table 3: Effect of combination of telmisartan with luteolin on transfer latency in elevated plus maze model in High-fat diet and streptozotocin-induced cognitive dysfunction in rats.

Na CMC- Sodium Carboxy methyl cellulose, NC- Normal control, DC- Dexamethasone control, PIO- Pioglitazone, TEL- Telmisartan, LUT- Luteolin, TL - Telmisartan plus Luteolin. Results are presented as mean ± SEM, (n=6). ANOVA followed by Dunnett test.

$^{\#\#\#}$p <0.001 when compared with NC. ***p<0.001, **p<0.01, *p<0.05 when compared with DC. ^{aaa}p<0.001, ^{aa}p<0.01, ^{a}p<0.05 when compared with TEL- I. ^{bbb}p<0.001, ^{bb}p<0.01, ^{b}p<0.05 when compared with TEL-II, ^{ccc}p<0.001, ^{cc}p<0.01, ^{c}p<0.05 when compared with LUT

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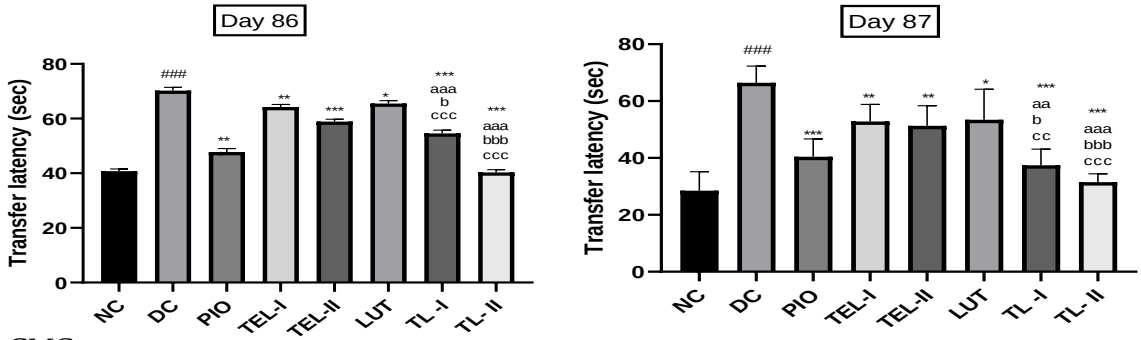

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[b]p<0.05 when compared with TEL-II, [ccc]p<0.001, [cc]p<0.01, [c]p<0.05 when compared with LUT.

Effect on GSH and LPO levels in brain

In the diabetic control (DC) group there was significant decrease (p<0.001) in GSH level, while significant increased (p<0.001) in LPO level normal control (NC) group. PIO treated groups showed significant increase (p<0.001) in GSH level, while significant decrease (p<0.001) in LPO level when compared with DC group. TEL-I treated group showed significant increase (p<0.05) in GSH level, while significant decrease (p<0.05) in LPO level when compared with DC group. TEL-II treated group showed significant increase (p<0.01) in GSH level, while significant decrease (p<0.001) in LPO level when compared with DC group. LUT treated group showed significant increase (p<0.001) in GSH level, while significant decrease (p<0.001) in LPO level when compared with DC group. TL-I and TL-II treated group showed significant increase (p<0.001) in GSH level, while significant decrease (p<0.001) in LPO level when compared with DC group. TL-I treated group showed significant increase in GSH level when compared with TEL-I (p<0.001), TEL-II (p<0.001) and LUT (p<0.05) group. TL-I treated group showed significant decrease in LPO level when compared with TEL-I (p<0.001), TEL-II (p<0.001) and LUT (p<0.05) group. TL-II treated group showed significant increase (p<0.001) in GSH level, while significant decrease (p<0.001) in LPO level when compared with TEL- I, TEL-II, LUT groups (Table 4 and Figure 4, 5).

Table 4: Effect of combination of telmisartan with luteolin on levels of GSH and LPO in brain of High-fat diet and streptozotocin-induced cognitive dysfunction in rats.

Groups	GSH (µmol/g of wet tissue)	LPO (nM of MDA/g of wet tissue)
NC	40.75 ± 1.16	3.87 ± 0.14
DC	16.13 ± 1.21[###]	9.14 ± 0.23[###]
PIO	37.67 ± 2.13[***]	4.98 ± 0.13[***]
TEL-I	18.62 ± 1.57[*]	8.68 ± 0.34[*]
TEL-II	20.12 ± 1.23[**]	8.47 ± 0.32[***]
LUT	23.25 ± 1.99[***]	7.54 ± 0.26[***]
TL-I	26.13 ± 1.12[***,aaa,bbb,cc]	6.83 ± 0.45[***, aaa,bbb,cc]
TL-II	34.75 ± 1.26[***,aaa,bbb,ccc]	4.96 ± 0.21[***aaa,bbb,ccc]

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[###]p <0.001 when compared with NC. [***]p<0.001, [**]p<0.01, [*]p<0.05 when compared with DC. [aaa]p<0.001, [aa]p<0.01, [a]p<0.05 when compared with TEL- I. [bbb]p<0.001, [bb]p<0.01, [b]p<0.05 when compared with TEL-II, [ccc]p<0.001, [cc]p<0.01, [c]p<0.05 when compared with LUT

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Figure 4: Effect of combination of telmisartan with luteolin on GSH level in brain of High-fat diet and streptozotocin-induced cognitive dysfunction in rats.

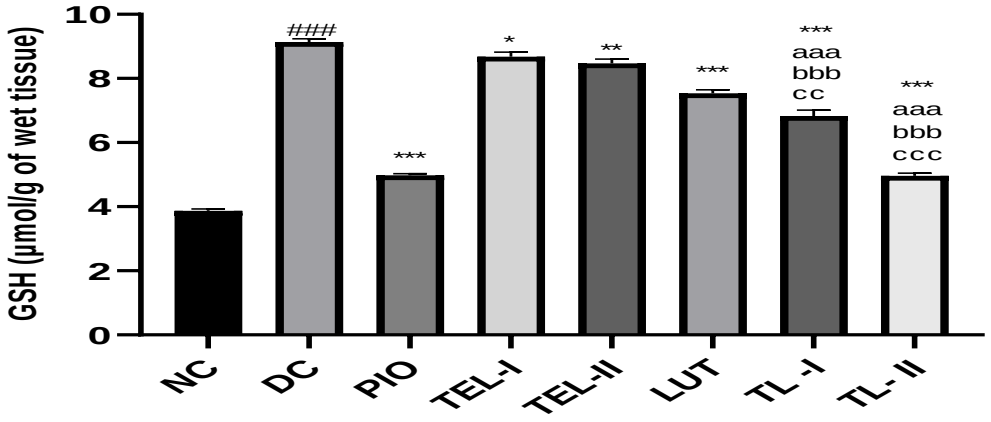

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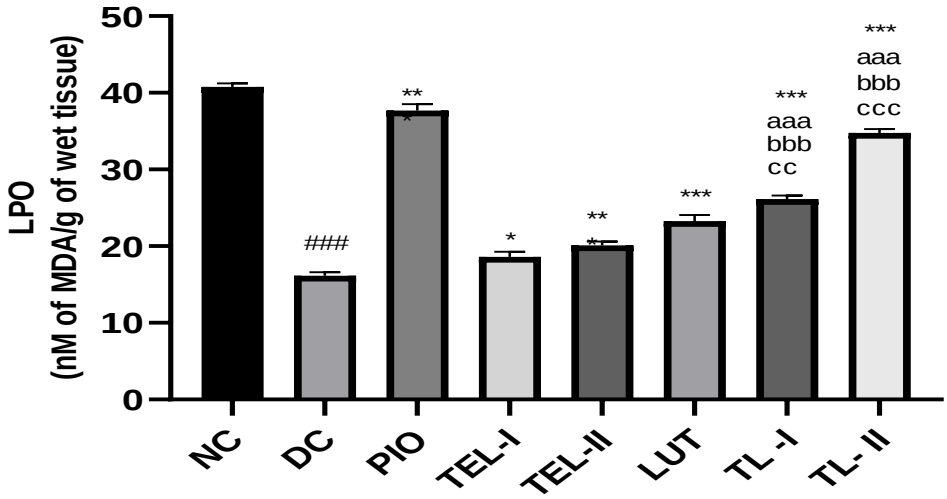
Figure 5: Effect of combination of telmisartan with luteolin on LPO level in brain of High-fat diet and streptozotocin-induced cognitive dysfunction in rats.



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$^{###}p$ <0.001 when compared with NC. $^{***}p<0.001$, $^{**}p<0.01$, $^{*}p<0.05$ when compared with DC. $^{aaa}p<0.001$, $^{aa}p<0.01$, $^{a}p<0.05$ when compared with TEL- I. $^{bbb}p<0.001$, $^{bb}p<0.01$, $^{b}p<0.05$ when compared with TEL-II, $^{ccc}p<0.001$, $^{cc}p<0.01$, $^{c}p<0.05$ when compared with LUT.

Effect on serum glucose level

DC Group treated with dexamethasone showed significant increase ($p<0.001$) in serum glucose level on 91 day onwards when compared with NC (normal control group). PIO Group treated with dexamethasone plus pioglitazone showed significant decrease ($p<0.001$) in serum glucose level on day 91 when compared with DC (dexamethasone control group). TEL– I Group treated with dexamethasone plus telmisartan (5mg/kg) showed significant decrease ($p<0.001$) in serum glucose level on day 91 when compared with DC (dexamethasone control group). TEL- II Group treated with dexamethasone plus telmisartan (10mg/kg) showed significant decrease ($p<0.001$) in serum glucose level on day 91 when compared with DC (dexamethasone control group). LUT Group treated with dexamethasone plus luteolin showed significant decrease ($p<0.001$) in serum glucose level on day 91 when compared with DC (dexamethasone control group), LUT (luteolin), TEL-I (telmisartan - 5mg/kg) and TEL-II (telmisartan 10mg/kg) treated groups. TL- II Group treated with dexamethasone plus telmisartan with luteolin at high lose i.e. telmisartan (10 mg) plus luteolin (10 mg) significant decrease ($p<0.001$) in serum glucose level on day 91 compared with DC (diabetic control), LUT (luteolin), TEL- I (telmisartan - 5mg/kg) and TEL- II (telmisartan 10mg/kg) treated groups (Table 5 and Figure 6).

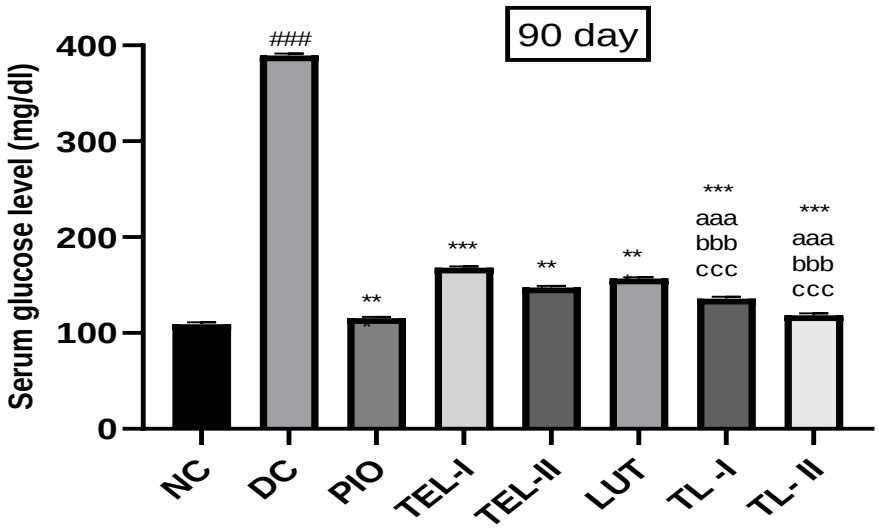
Table 5: Effect of combination of telmisartan with luteolin on serum glucose level in High-fat diet and streptozotocin-induced cognitive dysfunction in rats.

Group	Serum glucose (mg/dl) on	
	Day 1	Day 90
NC	106.56 ± 6.12	109.33 ± 4.78
DC	110.23 ± 5.62	389.52 ± 5.05$^{###}$
PIO	103.88 ± 5.24	115.44 ± 3.01***
TEL- I	104.87 ± 6.94	168.02 ± 3.25***
TEL- II	102.78 ± 4.76	147.54 ± 4.10***
LUT	105.42 ± 5.45	156.68 ± 3.87***
TL- I	104.29± 4.12	135.82 ± 4.96$^{***,\ aaa,bbb,ccc}$
TL- II	100.63± 5.23	118.57 ± 4.78^{***,aaa,bbb,ccc}

Na CMC- Sodium Carboxy methyl cellulose, NC- Normal control, DC- Dexamethasone control, PIO- Pioglitazone, TEL- Telmisartan, LUT- Luteolin, TL - Telmisartan plus Luteolin. Results are presented as mean ± SEM, (n=6). ANOVA followed by Dunnett test.

$^{###}p$ <0.001 when compared with NC. $^{***}p<0.001$, $^{**}p<0.01$, $^{*}p<0.05$ when compared with DC. $^{aaa}p<0.001$, $^{aa}p<0.01$, $^{a}p<0.05$ when compared with TEL- I. $^{bbb}p<0.001$, $^{bb}p<0.01$, $^{b}p<0.05$ when compared with TEL-II, $^{ccc}p<0.001$, $^{cc}p<0.01$, $^{c}p<0.05$ when compared with LUT.

Figure 6: Effect of combination of telmisartan with luteolin on serum glucose level in High-fat diet and streptozotocin-induced cognitive dysfunction in rats.



Na CMC- Sodium Carboxy methyl cellulose, NC- Normal control, DC- Dexamethasone control, PIO- Pioglitazone, TEL- Telmisartan, LUT- Luteolin, TL - Telmisartan plus Luteolin. Results are presented as mean ± SEM, (n=6). ANOVA followed by Dunnett test.
$^{###}p$ <0.001 when compared with NC. $^{***}p<0.001$, $^{**}p<0.01$, $^{*}p<0.05$ when compared with DC. $^{aaa}p<0.001$, $^{aa}p<0.01$, $^{a}p<0.05$ when compared with TEL- I. $^{bbb}p<0.001$, $^{bb}p<0.01$, $^{b}p<0.05$ when compared with TEL-II, $^{ccc}p<0.001$, $^{cc}p<0.01$, $^{c}p<0.05$ when compared with LUT.

Effect on body weight

DC Group treated with dexamethasone showed significant decrease ($p<0.001$) in body weight on day 90 when compared with NC (normal control group). PIO Group treated with dexamethasone and Pioglitazone showed significant increase ($p<0.001$) in body weight on day 90 when compared with DC (dexamethasone control group). TEL- I Group treated with dexamethasone plus telmisartan (5mg/kg) showed significant increase ($p<0.01$) in body weight on day 90 when compared DC (dexamethasone control group). TEL- II Group treated with dexamethasone plus telmisartan (10mg/kg) showed significant increase ($p<0.001$) in body weight on day 90 when compared DC (dexamethasone control group). LUT Group treated with dexamethasone plus luteolin showed significant increase ($p<0.01$) in body weight on day 90 when compared with DC (dexamethasone control group). TL- I Group treated with dexamethasone and telmisartan with luteolin at low lose i.e. telmisartan (5 mg) plus luteolin (10 mg) significant increase ($p<0.001$) in body weight on day 90 when compared with DC (dexamethasone control group). TL- I Group treated with dexamethasone and telmisartan with luteolin at low lose i.e. telmisartan (5 mg) plus luteolin (10 mg) showed significant increase in body weight on day 90 when compared with LUT ($p<0.001$), TEL- I ($p<0.001$) and TEL- II ($p<0.01$) treated groups. TL- II Group treated with dexamethasone and telmisartan with luteolin at high lose i.e. telmisartan (10 mg) plus luteolin (10 mg) showed significant increase ($p<0.001$) in body weight on day 90 when compared with DC

(dexamethasone control group). TL- II Group treated with dexamethasone and telmisartan with luteolin at high lose i.e. telmisartan (10 mg) plus luteolin (10 mg) showed significant increase ($p<0.001$) in body weight on day 90 when compared with LUT (luteolin), TEL- I (telmisartan - 5mg/kg) and TEL- II (telmisartan - 10mg/kg) treated groups (Table 6 and Figure 7).

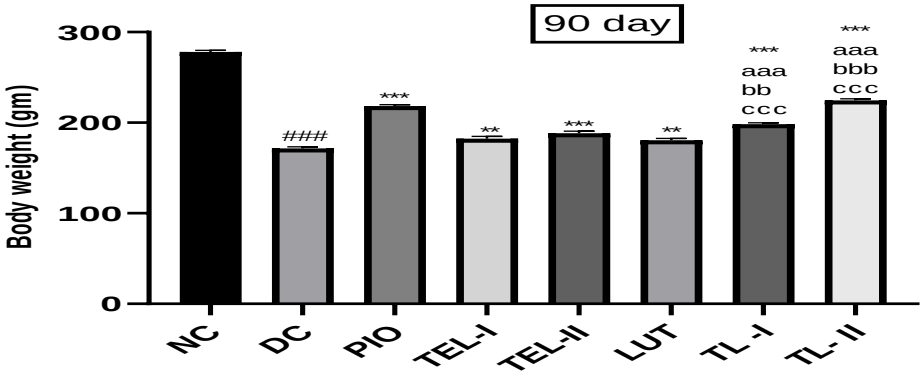
Table 6: Effect of combination of telmisartan with luteolin on body weight in High-fat diet and streptozotocin-induced cognitive dysfunction in rats.

Group	Body weight (gm)	
	Day 1	Day 90
NC	204.26 ± 5.05	278.14 ± 4.50
DC	209.12 ± 4.41	171.54 ± 4.24$^{###}$
PIO	201.42 ± 4.30	218.25 ± 4.25***
TEL- I	202.45 ± 5.52	182.46 ± 5.68**
TEL- II	207.32 ± 4.87	188.34 ± 5.31***
LUT	201.44 ± 4.58	180.67 ± 4.79**
TL- I	204.42± 4.34	198.44 ± 3.67$^{***,\ aaa,bb,ccc}$
TL- II	201.34± 4.65	224.68 ± 4.11^{***,aaa,bbb,ccc}

Na CMC- Sodium Carboxy methyl cellulose, NC- Normal control, DC- Dexamethasone control, PIO- Pioglitazone, TEL- Telmisartan, LUT- Luteolin, TL - Telmisartan plus Luteolin. Results are presented as mean ± SEM, (n=6). ANOVA followed by Dunnett test.

$^{###}p<0.001$ when compared with NC. $^{***}p<0.001$, $^{**}p<0.01$, $^{*}p<0.05$ when compared with DC. $^{aaa}p<0.001$, $^{aa}p<0.01$, $^{a}p<0.05$ when compared with TEL- I. $^{bbb}p<0.001$, $^{bb}p<0.01$, $^{b}p<0.05$ when compared with TEL-II, $^{ccc}p<0.001$, $^{cc}p<0.01$, $^{c}p<0.05$ when compared with LUT.

Figure 7: Effect of combination of telmisartan with luteolin on body weight in High-fat diet and streptozotocin-induced cognitive dysfunction in rats.



Na CMC- Sodium Carboxy methyl cellulose, NC- Normal control, DC- Dexamethasone control, PIO- Pioglitazone, TEL- Telmisartan, LUT- Luteolin, TL - Telmisartan plus Luteolin. Results are presented as mean ± SEM, (n=6). ANOVA followed by Dunnett test.
$^{###}p$ <0.001 when compared with NC. $^{***}p<0.001$, $^{**}p<0.01$, $^{*}p<0.05$ when compared with DC. $^{aaa}p<0.001$, $^{aa}p<0.01$, $^{a}p<0.05$ when compared with TEL- I. $^{bbb}p<0.001$, $^{bb}p<0.01$, $^{b}p<0.05$ when compared with TEL-II, $^{ccc}p<0.001$, $^{cc}p<0.01$, $^{c}p<0.05$ when compared with LUT.

Discussion

Cognitive decline brought on by oxidative stress-induced neurological impairment is a hallmark of prolonged hyperglycemia in diabetes. One of the main causes of age-related brain dysfunction in oxidative stress is a high rate of free radical formation lacking a corresponding degree of antioxidant defense. According to Gispen and Biessels (2002), the process of oxidative stress causes neurological damage that contributes to anatomical abnormalities, diminished memory, and long-term consequences (Bhutada et al., 2010).

Through destruction of DNA and cell membrane peroxidation of lipids, hyperglycemia generates free radicals, which lead to neuronal death (Hawkins and Davies, 2001). The activities of many enzymes, including those crucial to the healthy operation of the brain like catalase, 5'-nucleotide, and NTPDase, are altered as a result of the oxidative stress caused by hyperglycemia (Tuzcu and Baydas, 2006, Schmatz et al., 2009).

Oxidative damage to synapses in the rat brain's cortex and hippocampus is a factor in the impairment of cognitive functions. Antioxidants could therefore be helpful in preventing neurological damage and memory loss linked to hyperglycemia (Hasanein & Shahidi, 2010).

The presence of insulin receptors and insulin in the central nervous system may indicate its role in learning and recall (Zhao et al., 2004). Insulin signaling is compromised by oxidative stress (Hoyer and Lannert, 1999). Diabetic individuals have different brain electrophysiological and structural defects, which are additional compelling evidence that insulin resistance contributes to cognitive dysfunction (Gispen and Biessels, 2002). According to Maiese et al. (2007), individuals with diabetes have a doubled risk of developing cognition

Hyperglycemia may be due to the induction of insulin resistance in response to a high fat diet. However, the Randle cycle or glucose-fatty acid cycle is typically considered the primary mechanism involved. Partial destruction of Beta cells of pancreases by low dose of STZ also reason for developing hyperglycemia (Srinivasan et al., 2005; Correia-Santos et al., 2012).

Combination treated group tend to normalized levels of glucose may due to fact that telmisartan PPAR- Gamma partial agonist, enhance insulin sensitivity, stimulate glucose uptake in peripheral tissues such as adipose tissue, and induce the translocation of glucose transporter-4 to the plasma membrane. These effects are likely mediated through the activation of PPAR-gamma, which could help to maintain blood glucose levels. On the other hand, luteolin exerts its effects on adipose tissue

by reducing lipid peroxidation, enhancing the activity of antioxidant enzymes, limiting mast cell infiltration, reducing adipocyte hypertrophy, improving glucose uptake and insulin sensitivity, and inhibiting inflammation (Wang et al., 2021).

In current investigation, reduction in body weight in diabetic control group as compared to normal control. The reduction in weight may be result of decreased food consumption which also reported in many findings (Magalhães et al., 2019).

Comparing the telmisartan and luteolin treatment groups to the diabetic control group and the individual treatment groups, the telmisartan and luteolin combination treatment groups showed a statistically significant increase in body weight. The study's findings, which showed that luteolin significantly reduced weight loss in diabetic rats and effectively restored their weight to near-normal levels, may be used to explain the mechanism underlying the restoration of body weight in the combination groups. This finding shows that luteolin may increase protein synthesis and overall quality of life for diabetics by regulating blood sugar levels (Alam et al., 2023).

Baydas et al. (2006) suggested that the causes of cognitive impairments in individuals with diabetes may involve the generation of free radicals, as well as the accumulation of advanced glycation end products (AGEs) and the toxic effects of glucose resulting from chronic hyperglycemia. One of the main internal defense mechanisms is GSH, and it has been established that various neurological conditions are accompanied with a decrease in GSH. The central nervous system is protected from oxidative stress by a rise in spontaneous GSH levels brought on by dietary or pharmaceutical consumption of GSH precursors. Rats with diabetes who are not being treated had lower levels of GSH. In the cerebral cortex and hippocampus of diabetic rats, there is an increase in peroxidation of lipids and a decrease in glutathione, superoxide dismutase, and catalase activity (Kuhad and Chopra, 2007).

In the present investigation, combination of telmisartan with luteolin, showed significant increase in the brain GSH levels, and reduction in lipid peroxidation as compared to diabetic control as well as telmisartan low as well as high dose and luteolin treated groups. (Ohga et al., 2006). According to Heneka et al. (2000), Telmisartan may reduce LPO and increases GSH level may be due to increasing expression of HMGB1 in plasma by acting on the PPAR-gamma receptor. This effect could be attributed to several mechanisms, such as a decrease in endogenous nitric oxide activity, obstruction of NF-B, inhibition of TNF-release, or activation of PPAR agonists, all of which may help to mitigate the detrimental effects of oxidative stress. Also treatment with telmisartan causes reduction of protein expressions of NADPH oxidase sub-units can result in a decrease in oxidative stress (Lakshmanan et al., 2011). The administration of luteolin has been observed to induce changes in both the levels of glutathione and the activity of its metabolizing enzymes, which could potentially explain the increase in

GSH concentration observed in the brain homogenates of rats (Prasad et al., 2007).

Numerous recurrence of the Morris water maze task accompanied by reduced escape delay show that memory and learning continue to operate correctly. The results of the current study indicated that the performance of the diabetes control group on the Morris water maze task was impaired. The Morris water maze task, which assesses spatial memory, is based on the concept of reward, where rats attempt to locate a hidden platform in order to escape from swimming (Pathan et al., 2006). The findings of the present study demonstrated an association between the amount of malondialdehyde (LPO) in the brain and the time required to reach the concealed platform in rats with diabetes. Moreover, the final glucose levels in the study exhibited a negative correlation with glutathione levels and a positive correlation with MDA levels.

Takane et al. (2017) discovered that the human brain possesses its own renin-angiotensin system. Angiotensin II, known for promoting amyloid formation and triggering Alzheimer's-like phosphorylation of tau, plays a detrimental role in the progression of dementia. It exacerbates memory loss and cognitive decline, leading to increased neurological damage caused by amyloid.

Based on the current study, it can be inferred that the combination of telmisartan and luteolin improved scores on the Morris water maze task following 90 days of treatment. This improvement in cognitive function may be attributed to the ability of telmisartan and luteolin to reduce malondialdehyde (LPO) concentrations and increase glutathione (GSH) levels in the brain (Yu et al., 2015).

Additionally, telmisartan's ability to regulate glucose levels, suppress of apoptosis neurons which may have contributed to the alleviation of memory impairment. It lowers inflammation of neurons, possess free radical scavenging activity, improves amyloid elimination, and prevents phosphorylation of tau protein which may responsible (Huber et al., 2021).

To evaluate the extent of memory consolidation, a probe trial was conducted. The aim of the MWM probe exercise was to assess reference memory following an instructional session (Vorhees and Williams, 2006). The duration spent in the designated area following the training indicates the level of memory consolidation achieved. In current experiment combination treated group of telmisartan with luteolin showed significant memory retention as compared to diabetic control as well individual groups which may be due to decrease antioxidant, antihyperglycemic and neuroprotective effect of both.

The elevated plus maze assessment is recommended as a straightforward technique for evaluating memory and cognitive functions. Wistar rats demonstrate the ability to recall the arrangement of the open and enclosed arms, which enables them to escape the dangerous open arm more rapidly in subsequent trials. This test permits the evaluation of fear-motivated memory underlying the transfer latency method. The rat's reduced transfer latency during the second trial is utilized as an indicator of memory consolidation or recall.

In case of elevated plus maze, the transfer latency in the diabetic control group was significantly increased when compared to normal control group which shows that there was cognitive impairment in diabetic rats. Transfer latency was found to be significantly decreased in telmisartan plus luteolin treated groups as compared to diabetic control, which might be due to the glycemic control along with the potent antioxidant activity of the combination which was evident from the increased level of glutathione and decreased level of LPO in the telmisartan plus luteolin groups. The reason behind improvement in learning in combination treated groups telmisartan plus luteolin might be due to decrease in oxidative stress via antioxidant effect of luteolin which scavenges free radicals and restores normal levels of endogenous antioxidants as well as the effect of telmisartan to decrease the glucose toxicity induced oxidative stress and cellular damage (Huber et al., 2021, Kurata et al.,2015).

Conclusion

From present study it can be concluded that administration of telmisartan with luteolin shows synergistic effect in attenuating memory loss rats may be due to restoration blood glucose, brain antioxidants and body weights.

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