Research Article

# Land Caltrop *(Tribulus terrestris)* Fruit Extract Improves Learning, Memory and Cognitive flexibility in Streptozotocin-Nicotinamide induced Diabetes Animal Model

**Bheemesh Vangalapati[1], Poornima.A.Manjrekar[1*], Anupama Hegde[1], Sheetal Ullal[2]**
[1]Dept. of Biochemistry, Kasturba Medical College, Manipal University, Mangalore, India.
[2]Dept. of Pharmacology, Kasturba Medical College, Manipal University, Mangalore, India.
***Corresponding author's E-mail:** poornima.manjrekar@manipal.edu

**Accepted on:** 04-04-2016; **Finalized on:** 31-05-2016.

**ABSTRACT**

*T. terrestris* commonly known as Gokshura, Gokharu or Puncture vine belongs to the family Zygophyllaceae, widely distributed throughout India and traditionally it is used for treatment of many infectious diseases. Present study was designed to evaluate the effect of *T. terrestris* dry fruit extract in diabetes induced cognitive dysfunction. Diabetes was induced by Streptozotocin & Nicotinamide (STZ-NA, i.p. route). Animals were divided into 7 groups for comparing the activity of *T. terrestris* (150 & 300mg) against standard (Glibenclamide) & control groups. Blood glucose was measured by glucometer. Learning & memory was tested using Morris water maze. Time taken to reach the platform (escape latencies) by animals was noted from day 1 to 8. Probe trial was conducted on day 9 to record the time spent in the different quadrants. Glucose levels were significantly reduced in test and standard groups when compared to diabetic controls. *T.terrestris* and glibenclamide treated groups had decreased escape latencies in learning phase. During probe trial, test and standard treated groups spent significantly more time in the target quadrant with less entries into other quadrants on day 9 compared to untreated diabetic controls. Our findings confirmed that learning and memory was impaired in diabetic controls. This displays the link between hyperglycemia and cognitive deficit. *T.terrestris* treatment (150 & 300mg) along with having hypoglycemic effects in diabetic rats, revealed improvement in learning & memory. Glibenclamide treated group also showed equal improvement in learning and memory as it was observed by their performance during task. Hence, controlling the blood glucose levels in diabetes can delay cognitive impairment.

**Keywords:** Cognitive impairment, Diabetes mellitus, Diabetic neuropathy, Morris water maze, *Tribulus terrestris*.

## INTRODUCTION

Hyperglycemia in diabetes leads to impairment of learning and memory. Diabetes causes progressive cortical and subcortical atrophy in brain, referred to as diabetic encephalopathy. These structural changes affect higher mental functions leading to cognitive impairment and dementia[1]. Many studies support the relationship between diabetes and cognitive dysfunction. Insulin is best known for its involvement in the regulation of numerous brain functions including cognition, memory, and synaptic plasticity through complex insulin/insulin receptor (IR) signaling pathways. Insulin resistance in type 2 diabetes impairs signaling pathways of brain leading to various biochemical and histopathological changes[2]. These changes are linked to hyperglycemia induced oxidative / glycemic stress.

The brain is especially vulnerable to oxidative damage as a result of its high oxygen consumption rate, abundant lipid content, and relative paucity of antioxidant enzymes as compared to other tissues. Hyperglycemia reduces antioxidant levels and concomitantly increases the production of free radicals.

These effects contribute to tissue damage in diabetes mellitus[3]. Further trials are needed to find the exact mechanistic link and whether learning and memory impairment in diabetes patients may be prevented by the strict control of blood glucose and restoration of antioxidants.

Traditionally used *T. terrestris* (common name- Caltrop or Gokshura, Gokharu, belongs to the family Zygophyllaceae) is widely distributed throughout India. It is a popularly known aphrodisiac[4]. It is also used as diuretic, anthelmintic and in the treatment of many infections[5]. The aqueous extract of *T. terrestris* dry fruits have Hypolipidemic[6], hypoglycemic[7] & antioxidant activities[8].

Whether the additional antioxidant property, along with antidiabetic activity of *T. terrestris* can produce beneficial effects in diabetics with cognitive impairment is unknown. Hence the present study was designed to evaluate the beneficial effects of *T. terrestris* dry fruit extract in diabetes induced cognitive dysfunction in rats using the Morris water maze.

## MATERIALS AND METHODS

### Animals

All procedures were performed in accordance with institutional guidelines for animal research and were approved by Institutional Animal Ethics Committee, Kasturba Medical College, Mangalore, Manipal University, Karnataka. Albino Wistar strain either sex rats weighing 100±5grams were procured from Central Animal House, Kasturba Medical College, Mangalore. All animals were fed in an animal facility with a 12 to 12 hour light-dark cycle and were given the standard rat chow and water ad libitum.[9]

### Plant and Chemicals

*Tribulus terrestris* dry Fruit Aqueous Extract was procured from Indian Herbal Company, Bangalore, India. Streptozotocin and Nicotinamide were bought from Himedia Drug Company, India. Glibenclamide was bought from Cipla Company, Mumbai, India.

### Induction of diabetes

Experimental diabetes mellitus was developed in adult rats by administering Nicotinamide (NA) and Streptozotocin (STZ). Animals received intraperitoneal administrations of Nicotinamide - 25 mg/kg dissolved in normal saline 15 minutes before an administration of Streptozotocin- 50 mg/kg dissolved in 0.1M citrate buffer (pH 4.5)[10]. Only rats having a blood glucose above 250 mg/dL was chosen and divided into groups.

### Experimental Design

42 rats (24 diabetic rats and 18 normal control rats) were used for the study. Animals were divided into seven groups (n=6) and oral administration of plant extract and Glibenclamide started on 7$^{th}$ day after STZ-NA injection and continued for 30 days.

Group I- Normal controls treated with saline (NC)

Group II- Untreated Diabetic controls (DC)

Group III- Normal rats treated with *T. terrestris* (150mg/kg of body weight) (NC+TT150)

Group IV- Normal rats treated with *T. terrestris* (300mg/kg of body weight) (NC+TT300)

Group V- Diabetic rats treated with *T. terrestris* (150mg/kg of body weight) (DM+TT150)

Group VI- Diabetic rats treated with *T. terrestris* (300mg/kg of body weight) (DM+TT300)

Group VII- Diabetic rats treated with Glibenclamide 500µg (DM+Glib)

### Estimation of blood glucose

The test was done using ACCU-CHEK Active blood glucose monitor using disposable strips from Roche Diagnostics. Addition of 1-2µl of blood to the test pad gives the glucose values in 5-10 seconds.

### Morris Water Maze test

Test was started on 31$^{st}$ day after treatment & continued for 9 days. Spatial learning, memory and cognitive reflex was performed by the methods described by Papadopoulos[11] and Chen[12]. Day 1 to day 3 visible platform training & day 4 to day 8 hidden platform training was given.

Time taken to reach platform (TTP) by the rat was noted. On day 9 probe trial was conducted to test spatial memory by recording the time spent in different quadrants. Escape latencies and time spent in different quadrants was recorded by video camera.

### Statistical Analysis

The data is expressed as Median ± Standard Deviation (S.D.).

One way ANOVA and Independent t-test was used for between the groups analysis and Repeated measure ANOVA was used to analyze the repeated time intervals. SPSS Version 16 was used for analysis. p value < 0.05 was considered as significant.

## RESULTS

### *T.terrestris* Effect on Blood Glucose

After 30 days of treatment with the *T.terrestris* & Glibenclamide, the blood glucose levels was significantly ($p < 0.05$) reduced in STZ-NA induced diabetic rats compared to untreated diabetic controls (Table-1).

**Table 1:** Random blood glucose levels.*p value < 0.05

| Group | Day 1 (Median ± SEM) | Day 30 (Median ± SEM) | p value |
|---|---|---|---|
| NC | 118±2.8 | 123±3.5 | 0.40 |
| DC | 454±16.8 | 416±16.6 | 0.11 |
| NC+TT150 | 124±1.9 | 123±1.1 | 0.67 |
| NC+TT300 | 124±1.7 | 121±1.3 | 0.52 |
| DM+TT150 | 488±12.6 | 296±23.0 | 0.02* |
| DM+TT300 | 468±5.9 | 264±16.3 | 0.02* |
| DM+Glib | 451±19.22 | 200±18.41 | 0.01* |

### *T. terrestris* improved the spatial learning and memory-Target Quadrant

Escape latencies of various groups from day 1 to day 8 is shown in figure-1.

TTPs on Day 3 and Day 8 between the test compound against standard and controls groups is shown in Table-2. STZ-NA induced Diabetic control rats exhibited considerably increased escape latencies ($p<0.001$) in finding the platform compared to normal control group (Figure-2).

T. terrestris treated diabetic animals had significant decrease in escape latencies compared to diabetic controls (Figure-3).

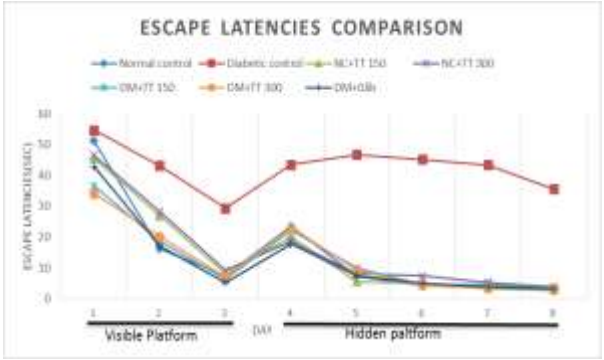


**Figure 1:** Escape latencies comparison of various groups

**Table 2:** Escape latencies on day 3 and day 8

| Group | On Day 3, Mean±S.D (TTP in Sec) | On Day 8, Mean±S.D (TTP in Sec) |
|---|---|---|
| NC | 7.50±1.97 | 2.63±0.79 |
| DC | 29.33±6.59 | 35.50±3.01 |
| NC+TT150 | 8.16±0.75 | 2.75±0.28 |
| NC+TT300 | 9.16±1.16 | 3.73±0.76 |
| DM+TT150 | 5.33±1.96 | 2.80±0.67 |
| DM+TT300 | 5.66±1.21 | 2.98±0.53 |
| DM+Glib | 5.08±0.80 | 3.16±0.40 |

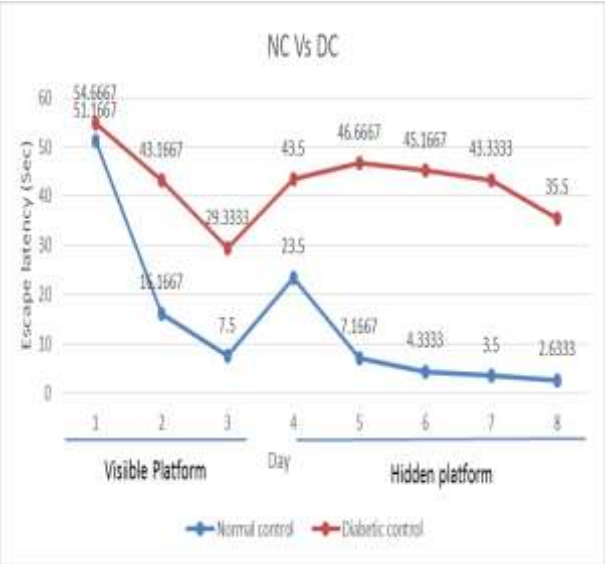


**Figure 2:** Escape latencies comparison between Normal Controls & Diabetic Controls

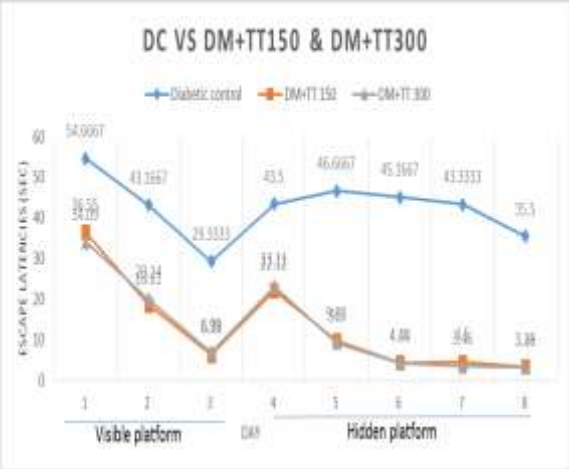


**Figure 3:** Escape latencies comparison between Diabetic Controls & Diabetic treated with *T.terrestris* 150mg & 300mg.

Diabetic group also had a significant ($p<0.001$) decreased inclination for the target quadrant (DC-13.32±3.63, NC-31.83±2.92, DM+TT150- 40.25±1.78, DM+TT300-42.41±3.61, DM+Glib- 30.91±5.71, NC+TT150-37.68±6.31, NC+TT300- 37.01±5.83 Sec) during the probe trial (Figure- 4).

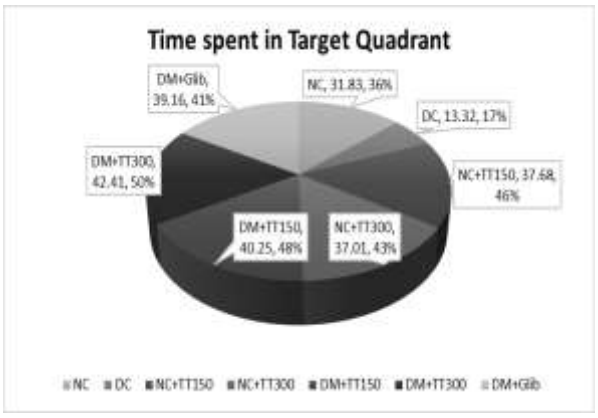


**Figure 4:** Time spent in Target quadrants (seconds & percentage)

***T.terrestris* effects on cognitive flexibility: Original quadrant results**

Diabetic rats spent significantly more time in the original quadrant than normal controls during the probe trial, (DC-40% & NC-19%, $p<0.001$) as shown in Figure-5. The preference of diabetic group was more for the visible platform quadrant (original quadrant) as compared to other quadrants, including the hidden platform quadrant.

Normal rats treated with *T.terrestris* (NC+TT150 & NC+TT300) showed decreased time spent in the original quadrant. Similarly, the *T.terrestris* and Glibenclamide treated diabetic rats also spent significantly less time (Figure-5) in the original quadrant. However, the search of treated group rats for the target quadrant improved slightly, but was not complete since *T.terrestris* & Glibenclamide treated rats explored the other quadrants also as observed by their movement pattern.

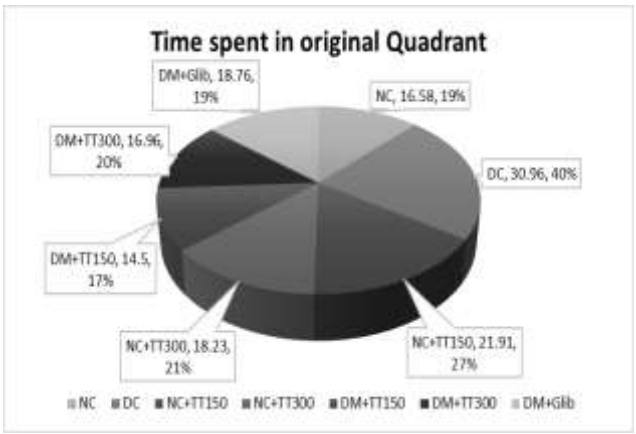


**Figure 5:** Time spent in Original quadrant (seconds & percentage)

## DISCUSSION

The present study shows that *T.terrestris* has favorable effects on cognitive function with improved learning, memory and adaptive capability. The study animals were able to face new and unexpected environmental changes after performing a task for some time (Cognitive flexibility). *T.terrestris* & Glibenclamide treated rats showed decreased escape latencies during learning

phase. During probe trial they focused on the target quadrant (quadrant with the hidden platform) with less entries into other quadrants. In contrast, untreated diabetic rats had increased escape latencies, difficulty in learning new task & spent less time in target quadrant during learning phase and on probe trial day respectively. These changes are suggestive of cognitive impairment in diabetic rats. Chronic hyperglycemia and insulin resistance associated cerebrovascular alterations may be the cause for these changes in diabetes animals.

Many studies have supported the fact that *T.terrestris* has positive effects on cognitive function in diabetic rats. A study done by Prabhu[13] explains the dose dependent beneficial effect of the aqueous extract of *T.terrestris* on learning and memory in rats. In another study oral feeding of *T.terrestris* improved learning and memory in STZ induced diabetic rats[14]. The studies were done using various behavioural models such as Hebb William Maze, T maze, Y maze and passive avoidance test. In the present study, there was statistically significant improvement ($p<0.001$) in learning and memory in *T.terrestris* treated study animal, by using Morris water maze behavioural model. High dose (300mg) of *T.terrestris* was found to be significantly ($p<0.001$) more effective compared to 150mg.

The beneficial effects of the plant extract have been attributed to its potent hypoglycemic effects which is evident by reduced blood glucose levels after 30days of treatment. In another study, *T.terrestris* methanolic extract treatment not only reduced blood glucose level but also significantly decreased glycosylated haemoglobin level[15]. The plant active compound, saponin from *T.terrestris* may be responsible for the hypoglycemic effects[7]. Interestingly, in Morris water maze task, Glibenclamide treated group also showed improvement in learning and memory as it was observed by their performance during task. This could be due to reduction in hyperglycemia induced stress in diabetes by hypoglycemic action of glibenclamide.

In diabetes, hyperglycemia induced oxidative stress releases numerous free radicals (through enzymatic and non-enzymatic pathways) which cause extensive tissue damage, neuronal degeneration & cognitive deficits. *T.terrestris* was found to have antioxidant activity, In our earlier studies the antioxidant potential of *T.terrestris* has been correlated with the presence of sufficient phenols[8]. Inhibition of lipid peroxidation in hippocampus reduced hippocampal cells apoptosis. This may contribute to its neuroprotective activity of *T.terrestris*[14].

Inflammasomes are involved in the secretion of proinflammatory cytokines in the brain. Increased cytokine secretion results in inflammation in the brain has been associated with cognitive impairment. Administration of *Rumalaya forte* which contains *T.terrestris,* attenuated motor in-coordination, inflammation and reversed alterations in levels of antioxidant enzymes[16]. Previous study findings suggest that extract of *T.terrestris* suppressed the expression of pro-inflammatory cytokines, such as TNF-α and IL-4 in macrophage cell line[17].

Hence, the reversal of cognitive deficit by *T.terrestris* could be due to its antidiabetic, antioxidant and anti-inflammatory properties which reduces the hyperglycemia induced stress related complications in diabetic animals.

## CONCLUSION

Impaired learning and memory was observed in diabetic animals. When treated with *T.terrestris* (150 & 300mg), along with hypoglycemic effects, there was dose dependent improvement in cognitive function by restoring spatial learning & memory activities.

Hence, controlling the blood glucose levels with the treatment of *T.terrestris* in diabetes can delay cognitive impairment.

## REFERENCES


1. Barbagallo M, Dominguez L J, Type 2 diabetes mellitus and Alzheimer's disease, World J Diabetes, 5(6), 2014, 889-93.
2. Zhao W Q, Alkon D L, "Role of insulin and insulin receptor in learning and memory," Mol Cell Endocrinol, 177, 2001, 125–34.
3. Bonnefont-Rousselot D, "Glucose and reactive oxygen species," Curr Opin Clin Nutr Metab Care, 5, 2002, 561–8.
4. Singh S, Nair V, GuptaY K, Evaluation of the aphrodisiac activity of *Tribulus terrestris* Linn. in sexually sluggish male albino rats, Journal of Pharmacology and Pharmacotherapeutics, 3, 2012, 43-7.
5. Tantawy W H E I, Hassanin L A, Hypoglycemic and hypolipidemic effect of alcoholic extract of *Tribulus alatus* in streptozotocin-induced diabetic rats; A comparative study with *T. terrestris* (caltrop), Indian journal of experimental Biology, 45, 2007, 785-90.
6. Chu S, Su W, Sun B, Huang X, Effect of saponins from *Tribulus terrestris* on hyperlipidemia, Zhong Yao Cai, 26, 2003, 341.
7. Li M, Wang Y, Tian C, Hypoglycemic effects of saponin from *Tribulus terrestris*, Zhong Yao Cai, 25, 2002, 420.
8. Vangalapati B, Manjrekar PA, Hegde A, Total phenolic content and free radical scavenging activity of *Pterocarpus marsupium* heartwood & *Tribulus terrestris* dry fruits: An *in vitro* comparative study, Journal of pharmacy research, 8(5), 2014, 610-3.
9. Committee for the purpose of control and supervision of experiments on animals, CPCSEA guidelines for laboratory animal facility, Indian journal of pharmacology, 35, 2003, 257-74.
10. Rabbani S I, Devi K, Khanam S, Protective role of glibenclamide against nicotinamide-streptozotocin induced nuclear damage in diabetic Wistar rats, Journal of pharmacology and therapeutics, 1, 2010, 18-23.
11. Papadopoulos P, Rosa-Neto P, Rochford J, Hamel E, Pioglitazone improves reversal learning and exerts mixed

cerebrovascular effects in a mouse model of Alzheimer's disease with combined Amyloid-β and cerebrovascular pathology, Journal.PLos One, 8(7), 2013, e68612.

12. Chen G, Chen K S, Knox J, Inglis J, Bernard, A learning deficit related to age and beta-amyloid plaques in a mouse model of Alzheimer's disease, Nature, 408, 2000, 975-9. doi:10.1038/35050103. PubMed: 11140684.
13. Prabhu N, Hadigal S, Sheetal U D, Sushma D S, Shenoy A K, Effect of *Tribulus Terrestris* on Learning and Memory in Wistar Rats, Phcog J, 06, 2014, 68-71.
14. Roghani M, Malayeri OS, Malayeri S, Effect of *T.terrestris* oral feeding on learning and memory in streptozotocin diabetic rats: Investigating the role of lipid peroxidation, Journal of guilan university of medical sciences, 22(85), 2013, 88-95.
15. Chu S, Su W, Sun B, Huang X, Effect of saponins from *Tribulus terrestris* on hyperlipidemia, Zhong Yao Cai, 26, 2003, 341.
16. Santhrani T, Vineela S, Ameliorative effect of *Rumalaya forte* in sciatic nerve transection induced neuropathy in rats, J Pharm Boil Res, 1(2), 2011, 136-44.
17. Oh JS, Baik SH, Ahn EK, Jeong W, Hong SS, Anti-inflammatory activity of Tribulus terrestris in RAW264.7 cells, The journal of Immunology, 188, 2012, 54.

**Source of Support:** Nil, **Conflict of Interest:** None.