ABSTRACT

Introduction
Diabetes Mellitus is not only a cluster of metabolic dysfunctions but is also related with dysfunction of cognition referred as “Diabetes encephalopathy”. Around 318 million adult populations are with impaired glucose tolerance or prediabetes state. In India alone 69.2 million people are diabetic and it is predicted that by the year 2040 this number might rise to 123.5 million. Despite that the first record of diabetic encephalopathy was reported as diabetes related cognitive dysfunction way back in 1922 till today the exact mechanism leading to this decline remains a mystery. Diabetes in both the forms is related with adverse brain changes and cognitive decline. Even with a good control of diabetes there are reports of mild cognitive impairments (MCI) in type 2 diabetic patients (T2DM). It has been documented that abnormalities of altered neurogenesis, neurochemistry, electrophysiological deficits, oxidative stress injury, neuroinflammation, and neuronal apoptosis can induce structural changes and could be involved in brain dysfunction during diabetes. Thus, it demands a medical attention for prevention and management. Although there is certain pharmacological treatment for cognitive dysfunction but not all come without adverse side effects. Luckily there are dietary nutrients that have the potential to revert or delay the cognitive dysfunction. One such important nutrient is the omega-3 PUFA, which are present in high concentration in fish oil, flaxseed oil, walnuts, chia seeds, canned fish, fresh sea fishes like mackerel, salmon, menhaden, sardine, roasted soybeans, spinach (boiled /cooked) etc. Dietary omega-3 PUFA has been found to improve learning and memory in normal as well as in neurodegenerative diseases. Their protective effect and neurogenic effect has been widely documented. Diabetes of both the types are known to be deficient with vitamin B_{12} and C, in the present study synergistic effect of supplementation with omega-3 PUFA rich fish oil and flaxseed oil and vitamin C and B_{12} has been studied and reported.

Aims and Objectives
To study the effect of supplementation of omega-3 PUFA rich fish oil and flaxseed oil in low dose and high dose on learning and memory, oxidative, antioxidant and inflammatory status in STZ induced diabetic rats.

To study the synergistic effect of supplementation of fish oil or flaxseed oil and vitamin C and vitamin B_{12} on learning and memory, oxidative and inflammatory status in STZ induced diabetic rats.
To compare the effects of omega-3 PUFA from animal source and plant source on STZ induced diabetic rats.

To compare the different dose effects of omega-3 PUFA rich fish oil and flaxseed oil on STZ induced diabetic rats.

To correlate the association of dietary supplementation and cognition in STZ induced diabetic rats.

Significance of the study

Individuals suffering from diabetes induced cognitive dysfunction and having low habitual intake of omega-3 PUFA may benefit most from the outcome of the study.

Materials and Methods

2-3 months Wistar male rats having a body weight of 200-250 gm was taken for the study. Approval of Institutional Animal Ethical Committee (IAEC) was obtained before the experiments were started (letter dated 2/02/2013). Fish oil and flaxseed oil was administered in two different doses and in combination of vitamin B<sub>12</sub> and vitamin C. Each group had 8 animals each and control groups were run parallel. Diabetes was induced by injecting STZ intraperitoneally @ 48 mg / kg body weight. Dietary supplementation was administered orally. After 30 days of treatment the animals were tested for different cognitive parameters using MWM, EPM, OFT and PAT followed by decapitation and preparation of brain tissue homogenates and collection of blood by cardiac puncture. The tissue homogenate was prepared in 1X phosphate buffered saline (PBS) with dilution 1:10 w/v and stored at -20 °C until analysed.

Biochemical estimations were performed for assessing the brain oxidative and antioxidant status in areas like hippocampus, frontal cortex and cerebellum. Hippocampal tissue homogenate was also tested for the BDNF and TNF-alpha levels.

Statistical Analysis

Data was analyzed by using statistical software IBM SPSS Statistics, Version 24.0. (Armonk, NY: IBM Corp.). All the results are expressed as Mean ± S.E.M. Graphs were obtained using GraphPad Prism Version 7.0 a and Microsoft® Excel for Mac, Version 15.28. Data analyzed by t-test and one-way ANOVA Data followed by post hoc using Tukey’s HSD test. Data that were not normally distributed were assessed by Kruskal Wallis H test followed by multiple comparison by Dunn’s test.
Results

Body weight, random blood sugar and brain weight results

There was reduction in body weight and hyperglycemic state was maintained throughout the study period.

Behavioral test results

- Morris water maze test: Repeated measure ANOVA showed a significant reduction in escape latency with training in the supplemenated group of animals. Diabetic control rats had impaired spatial learning and memory which gets rectified with dietary supplementation.
- Passive avoidance test: Diabetic rats showed poor memory retention and entered the dark chamber whereas the treated group had improved memory retention with increased latency to enter the dark chamber.
- Open Field test: Ambulation is decreased in diabetic control. Supplementation increases the locomotion.
- Elevated plus maze test: Untreated diabetic rats had increased anxiety and immobilization. Supplemented groups had reduced anxiety.

Biochemical test results

- Lipid profile results: Treatment with omega-3 PUFA and Vitamins improved the HDL levels and decreased the TG and TC levels in the diabetic rats.
- Lipid peroxidation results: A significant reduction in MDA levels in the brain tissue homogenates was observed in the treated group of animals.
- Protein Carbonyl content: Dietary treatment reduced the protein content in the diabetic rats.
- Reduced Glutathione levels in different brain regions: Significantly improved in the treated group of animals
- Total antioxidant capacity in different brain areas as assessed by Ferric Reducing Antioxidant Power (FRAP) assay: There was significant increase in the FRAP values in treated group of animals.
- Hippocampal brain derived neurotrophic factor (BDNF): Treated animals had significantly higher levels of BDNF as compared to the untreated.
- Hippocampal tumour necrosis factor-alpha (TNF-α): There was significant reduction in TNF-α levels in the brain tissue homogenate in the treated group of animals.
Conclusions

- Diabetic control rats showed impaired spatial learning and memory.
- Treatment with omega-3 rich fish oil and flaxseed oil alone and in combination with vitamin C and B₁₂ decreased the escape latencies of the rodents, indicating a beneficial effect on the spatial memory in the diabetic rats.
- No significant difference in behavioral test results in between the fish oil treated groups and flaxseed oil treated groups.
- There was no additive improvement in the cognition in the high dose group of animals in comparison to low dose administered group.
- There was increase in the antioxidant status in the diabetic rats.
- Positive correlation of protein carbonyl contents and the time taken to reach the target quadrant during probe trial of Morris water maze showing impaired memory.
- Correlation between the antioxidant levels in the different brain areas and cognitive function was observed. Higher the levels of antioxidants better were the performance.
- Supplementation with omega-3 alone and in combination with vitamin C and vitamin B₁₂ increased the hippocampal brain derived neurotrophic factor (BDNF).
- There was a reduction in the diabetes induced neuroinflammation in the hippocampal region on treatment with omega-3 rich oils and vitamins.
- There was a negative correlation between TG and memory performance as assessed by the time spent in the target quadrant during probe trial of Morris water maze test.
- HDL level was negatively correlated with time taken to reach the target quadrant and positively with the time spent in the target quadrant.
- Linear regression test indicated TG to be a better predictor for the time spent in the target quadrant.

Keywords: Diabetes, STZ, MWM, PAT, OFT, EPM, Oxidative status, BDNF, TNF-a