CONCLUSION AND SUMMARY

The followings were the conclusions of the present study:

➢ Male Wistar rats induced with diabetes showed impaired results in cognitive tasks. There was increased latency during training and probe trial sessions of Morris water maze test. The untreated rats failed to learn and memorize the escape pathway and thus their escape latencies were significantly increased. Treatment with omega-3 rich fish oil and flaxseed oil only and combination with vitamin C and B12 decreased the escape latencies of the rodents, thus indicating a beneficial effect on the spatial memory in the diabetic rats. Probe trial parameters like the time taken by the animal to reach the target quadrant and the time it spent in the target quadrant were taken as the standard parameter of cognitive function in rats during correlation analysis. There was no significant difference in the latency during training sessions in between the fish oil treated groups and flaxseed oil treated groups, which proves that the omega-3 from plant source had an equal effect as that of animal source. An additive improvement with high dose administration of fish oil and flaxseed oil in diabetic could be established in our study.

➢ Untreated diabetic rats had poor memory retention during passive avoidance test as they entered the dark chamber where foot shock was given on day 1 of passive avoidance test. The treated group of animals had better memory retention and took longer time or did not enter (fish oil high dose & flaxseed high dose + vitamin C group) the dark chamber at all. Untreated diabetic control rats spent lesser time in the bright chamber of passive avoidance apparatus, unlike the rats supplemented with omega-3 rich oils and vitamin C and B12.

➢ A significant reduction in locomotion was recorded in the diabetic animals during open field test. Animals remained immobilized for longer time and did not explore the central portion of the open field apparatus, indicating reduced motor activity in diabetic rats.

➢ In elevated maze plus there was reduction in the open arm entries and percentage of time spent there. Omega-3 PUFA supplementation reduced the anxiety in the diabetic rats as reflected by the increase in the exploration time.
in the open field test in treated group, the findings are in cohort with other study on restraint stress. Thus, omega-3 PUFA has anxiolytic effect on STZ induced diabetic rats.

- Hyperglycemic state was persistent throughout the study and the animals lost their body weight along the time course of study period. Although there was no significant change in the absolute cerebral cortex weight, but a significant change in absolute and relative weights of cerebellum, frontal cortex and hippocampus was observed. Since treatment with dietary supplementation had no body weight gain effect, the relative weight of the brain parts remained higher.

- Pathophysiology of cognitive decline in STZ induced diabetic rats is multifactorial. In the present study, we considered the brain oxidative status and tried correlating it with the cognitive dysfunction. A significant increase in MDA levels in the hippocampus, cerebellum and frontal cortex of the diabetic rats was observed as compared to the controls, highest being in the hippocampus. This could be one of the reasons for the impaired spatial memory in diabetic rats, which on supplementation with omega-3 and vitamins gets improved as evident from the results of treated group of rats. There was a positive correlation of protein carbonyl contents and the time taken to reach the target quadrant during probe trial of Morris water maze (MWM). More was the protein carbonyl content longer was the latency to reach the target quadrant demonstrating impaired spatial memory in the diabetic rats. Fish oil and flaxseed oil along with vitamins significantly reduced the protein carbonyl content.

- Diabetes in the experimental animals decreased the antioxidant levels in the different parts of the brain. There was reduction in the levels of glutathione and total antioxidants in the tissue homogenates of hippocampus, frontal cortex and cerebellum. Dietary supplementation of omega-3 and vitamins significantly improved their levels in the brain. In our study, we found a correlation between the antioxidant levels in the different brain areas and cognitive function. Higher the levels of antioxidants better were the performance.
➢ Supplementation with omega-3 only and combination with vitamin C and B₁₂ increased the hippocampal brain derived neurotrophic factor (BDNF). Untreated diabetic rats had reduced hippocampal BDNF levels which were negatively correlated with the time taken by the animal to reach the target quadrant during probe trial of MWM.

➢ There was a reduction in the diabetes induced neuroinflammation in the hippocampal region on treatment with omega-3 rich oils and vitamins. In the present study, untreated diabetic rats had higher value of tumor necrosis factor-alpha (TNF-α) which gets reduced on treatment. There was also a negative correlation between hippocampal TNF-α and cognitive parameter of Morris water maze. Animals with higher values of TNF-α had lesser memory retention and spent less time in the target quadrant during probe trial of MWM test. We also observed a relationship between TNF-α and BDNF, rats with higher proinflammatory marker in the hippocampus had lesser BDNF in the hippocampus. The results are in accordance with other studies.

➢ There was alteration in lipid profile in diabetic animals with high triglyceride (TG) and total cholesterol levels and low HDL level, which gets rectified on dietary supplementation.

➢ We found a new outcome in our study where we observed a correlation between lipid profile and cognition. There was a negative correlation between TG and memory performance. HDL level was negatively correlated with time taken by the animal to reach the target quadrant and positively with the time the animal spent in the target quadrant. Linear regression test indicated TG to be a better predictor for the time spent in the target quadrant. Thus, we can also state that elevated TG levels in diabetes could be another adding factor for cognitive dysfunction. Treatment with omega-3 PUFA and vitamins improved the HDL levels and decreased the TG and TC levels in the diabetic rats and enhanced the cognition in diabetic rats as seen in the behavioural test performances.
SUMMARY OF MECHANISMS OF OMEGA-3 PUFA AND VITAMIN C AND VITAMIN B\textsubscript{12}  
Mentioning the various beneficial effect of dietary supplementation of omega-3 PUFA and vitamin C and B\textsubscript{12} on STZ induced diabetic rats, it would be rationale to use it as a neuroprotective diet for the diabetic population. The neuroprotective properties of the supplementary diet are supported by its ability to maintain the lipid profile, improve the antioxidant levels and decrease the oxidative stress levels in the brain. The contribution of the dietary supplementation of omega-3 rich oils and vitamins on cognitive function is supported by its ability to increase the expression of BDNF in the hippocampus and reduce the neuroinflammation by decreasing the brain TNF-\(\alpha\).

PROSPECTIVES  
Potential prospect for this research deserves a further elucidation of the role of long term supplementation of omega-3 PUFA by conducting molecular based studies where the exact mechanism of action could be studied and the findings could be utilized in prevention of cognitive impairment in diabetic patients. There is also a need for clinical trial studies to clarify the neuroprotective function in the diabetic patients of both the types. Although several of clinical trials are evaluating the effect of omega-3 PUFA on cardiovascular diseases, very few have the cognitive changes as a major endpoint (563–566). With high prevalence of diabetes across the world, cognitive impairment in diabetic patients will also be in rise, making it more crucial.