

Investigation Of Antioxidants, Antidiabetic And Antihyperlipidemic Activity Of Vitex Trifolia Seeds In Diabetic Zebrafish Model

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DOI: 10.47750/pnr.2023.14.502.101

Abstract

Diabetes and obesity are now regarded as global epidemics. Researchers coined the term "diabesity" to describe the relationship between these two diseases as obesity and diabetes rates rise simultaneously. Type 2 diabetes mellitus, heart disease, and numerous types of cancer are all made more likely by obesity. Diabetes and obesity are two examples of complex diseases that are influenced by both hereditary and environmental factors. Due to the lack of knowledge regarding the underlying pathophysiology of obesity and diabetes, additional research is needed before pharmacological and surgical treatments can be used. Therefore, it is essential to use animal models of diabetes and/or obesity to advance our understanding of these conditions as well as to find and create efficient treatments for them. Zebrafish is a desirable model system for studying metabolic diseases due to functional conservation in lipid metabolism, adipose biology, pancreas structure, and glucose homeostasis. Finding new approaches to prevent and treat human diseases like diabetes and obesity is a good fit for it. This review discusses the advantages and disadvantages of using zebrafish as a model to study the pathologies associated with obesity and diabetes. It focuses on studies that have used zebrafish as a model for these diseases.

Keywords: Diabetic, Zebrafish, Obesity, Herbal plant

Introduction

One of the most prevalent and enduring diseases in human history is type 1 diabetes (DM). It was mentioned for the first time in an Egyptian book about 3000 years ago. Researchers first distinguished between type 1 and type 2 diabetes in 1936^[1,2]. Diabetes of the type 2 variety was recognised as a sign of the metabolic syndrome in 1988. The

most prevalent type of DM is known as non-insulin dependent DM, and its hallmarks include hyperglycemia, insulin resistance, and relative insulin insufficiency. Type 2 diabetes is brought on by a combination of environmental factors, genetic factors, and lifestyle factors^[3,4]. Due to the increased risk of both short-term and long-term complications, patients with type 2 diabetes frequently pass away at a young age. Patients with type 2 DM are more likely to experience complications and pass away since the condition is so common, advances so slowly, and is frequently misdiagnosed in underdeveloped regions like Africa. The WHO estimated that 31 million people in India had diabetes in 2000 (WHO). The world's population may total 79 million by 2030^[5]. Diabetes is treated with oral hypoglycemic medications, however these may have unfavourable side effects on the body, including as haematological consequences and alterations to the function of organs like the liver and kidneys. There are currently no effective long-term treatments for diabetic neuropathy. However, symptomatic therapy hasn't made much progress. The global hunt for medicinal plants with potential to cure diabetes has been covered in a wide range of recent articles. The WHO has suggested using traditional herbs as a safe and efficient treatment for diabetes mellitus. ^[6] People have used medicinal plants for thousands of years to find relief from a variety of medical conditions. *Trifolium* of *Vitex* A deciduous shrub known as *L. var. trifolia* (*V. trifolia*) is indigenous to the Pacific coasts of Asian nations like China, India, Australia, and Singapore ^[6,7]. There is a long history of using *Vetiveria trifolia* to treat inflammatory diseases. Other names for this plant include panikisanbhalu (Hindi), three-leaf chaste tree (English), and chaste tree (Latin) (in Chinese). The dried ripe fruits of *V. trifolia*, also known as *Fructus viticis*, have been used successfully in traditional Chinese medicine to treat a variety of ailments, including ocular inflammation, headache, impaired vision, rhinitis, and the common cold ^[8,9]. Traditional medicine from the Pacific uses seeds from the *V. trifolia* plant to treat inflammation and ciguatera fish poisoning ^[10]. A poultice helps relieve the pain of sprains and rheumatism, while a decoction produced from the seeds may soothe mouth irritation ^[11]. The stems are used to treat the symptoms of a fever with vomiting and thirst, while the infusion made from the flowers is used orally to treat diarrhoea ^[12]. According to certain sources, the roots are antiemetic, expectorant, and fever-reducing ^[13, 14]. Analysis of seed extracts from *V. trifolia* revealed the presence of terpenes including eucalyptol and caryophyllene ^[17], as well as flavonoids such casticin ^[15], vitexin ^[16], and luteolin. Scientists have validated the anti-inflammatory properties of *V. trifolia* leaf extracts using rat (using the Carrageenan-induced paw edoema rat model) and mouse models of inflammation (e.g., RAW264.7 mouse cell lines generated with lipopolysaccharide ^[18]). These studies examined leaf extracts prepared by decoction, Soxhlet, and maceration in ethanol and water. The inflammatory effects of *V. trifolia* on human macrophages and the range of solvents that can be employed to extract the plant's active ingredients are not well understood. Human monocytic cell lines like U937 can be used to develop into macrophages using inflammatory stimuli like phorbol 12-myristate 13-acetate (PMA), which can subsequently be used to study macrophage activity ^[19,20]. *V. trifolia* was one of the fresh medicinal herbs that was widely used in Singapore, as we had previously described in an ethnobotanical study ^[21]. Based on the plant's leaf extracts, which potentially inhibited the proliferation of many cancer cell lines, this usage was made ^[22]. The objectives of this work were to assess the effects of these extracts on cytokine production in PMA-differentiated U937 macrophages and isolate and characterise phytoconstituents from the most potent leaf extract of *V. trifolia*^[23]

Material & Methods

We search from different source Web of science, Scopus, NCBI, Google scholar, Keywords: plant, zebra fish, hyper lipidemic and Anti diabetic activity

Plant Extraction

Collection of Plant Materials:

Plant Extract Preparation

The aqueous extract was made using cold maceration. One hundred grammes of dry, coarse powder was mixed with one hundred millilitres of water, macerated for six hours, and then left at room temperature, intermittently shook, for eighteen hours. Arrange on a dish and dry in a pan of evaporating water. ^{[24][25]}

Preliminary Phytochemical Screening:

A phytochemical examination of an aqueous extract of *V. trifolia* seeds revealed the presence of steroids, sugars, proteins, amino acids, triterpenoids, alkaloids, tannins, flavonoids, and members of the phenolic chemical family. ^{[26][27]}

Animals:

Primary adult zebrafish were used in this investigation, and their only purpose was to reproduce. Three adult female wildtype zebrafish and three mature males would generally be found in a breeding tank. We separated the sexes with a solid wall to make sure the progress was timed correctly. We didn't begin construction until the following morning. When embryos were located in the bottom of the tank after the divider was removed, they were strained out using a strainer and placed on 60 mm diameter petri dishes, each dish having less than 50 embryos. Embryos are generally merely one cell at this stage. Over and over again, embryos are checked under a microscope to ensure they are healthy and viable. ^[28]

In vitro glycation BSA

The usual method for glycating BSA was modified slightly and was successfully carried out. In a nutshell, for four weeks at 37 °C, bovine serum albumin (BSA) (10 mg/mL) was incubated with glucose or fructose (0.5 M) in 0.1 M phosphate buffer (pH 7.4) with or without MOE (0.5-2.0 mg/mL) and aminoguanidine (AG, 1.0 mg/mL). Until analysis, the samples were stored at 20 degrees Celsius. ^[29]

In vivo Fish Model

Acute Toxicity of Methanolic Extract of *Vitex trifolia* L

The toxicity analysis was performed in accordance with the OEC's standards (OECD). Batch additions of 8 fish were made to 5 L tanks with 24±2 °C water and continual aeration. The batches were divided into three groups: In the first batch, there is only plain water; in the second batch, there is water plus 32 mg/L CSM; in the third batch, there is water plus 64 mg/L CSM; in the fourth batch, there is water plus 80 mg/L CSM; and in the fifth batch, there is water plus 5 mg/L 3,4 dichloroaniline (positive control). To maintain a steady concentration, the CSM extract and 3,4-dichloroaniline were continually swapped every 24 hours. The fish from the various batches did not eat during the 96 hours when the aforementioned conditions were kept up. The fish were routinely examined to gauge their swimming patterns, gill activity, and the occurrence of any deaths throughout the test's development. ^[30,31]

Glucose inhibits haemostasis and accelerates diet-induced hyperlipidaemia in zebrafish larvae

Numerous clinical features of diabetes in mammals are assumed to result from microvascular damage brought on by hyperglycemia. These characteristics include modifications to leukocyte biology, changes to hemostasis, and an accumulating number of macrophages that are lipid-rich in the vessel wall. Studies on mammals suggest that hyperglycemia-induced microvascular damage lowers the production of endothelium adhesion molecules necessary for immune cell recruitment. As a result, diabetic wounds do not attract neutrophils and macrophages as frequently. Due to the inflammatory nature of the wound environment, macrophages and neutrophils that do reach a diabetic lesion often assume an inflammatory phenotype. ^[32,33,34]

Administration of Methanol Extract of *Vitex trifolia* L:

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Result & Discussion

An initial phytochemical analysis of the seeds found a variety of phytoconstituents, including carbohydrates, phenolic compounds, flavonoids, proteins, amino acids, tannins, phytosterols, and saponins the results were shown.

Table 1: Preliminary Phytochemical Screening of Vitex trifolia

Constituents	Test	P. ether	Ethanol	Aqueous
Alkaloids	Mayer Reagents	+	+	+
	Drangondorff Reagents	+	-	-
	Hager Reagents	-	+	+
	Wagner reagents	-	-	+
Carbohydrate test	Molish test	+	-	+
	Barfoed test	+	+	-
	Fehling test	+	+	-
	Benedicts test	+	+	-
Phenolic compounds	Ferric Chloride	-	+	+
	Lead acetate	+	+	-
Flavanoids	Shinods Test	+	-	+
	Conc . H ₂ O	-	-	-
	NaOH test	+	+	+
Protein	Biuret test	-	-	-
	Ninhydrin test	+	+	+
Tannins	Ferric chloride	+	+	-
	Potassium dichromate	+	+	+
Saponins	Foam test	+	-	+

Fixed oil & fats	Spot test	+	+	+
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In vitro glycation BSA

High levels of fluorescence are indicative of the onset of advanced age. Figure 2 displays BSA/glucose system fluorescence after 1, 2, 3, and 4 weeks of incubation. Incubated samples lose fluorescence intensity as BSA, glucose, and CSM concentrations increase. After incubation for 4 weeks, the concentration of 5 mg/mL showed the highest decrease (90.45%) compared to glycated BSA. The BSA/glucose/AG control system demonstrated a 91.8% decrease.

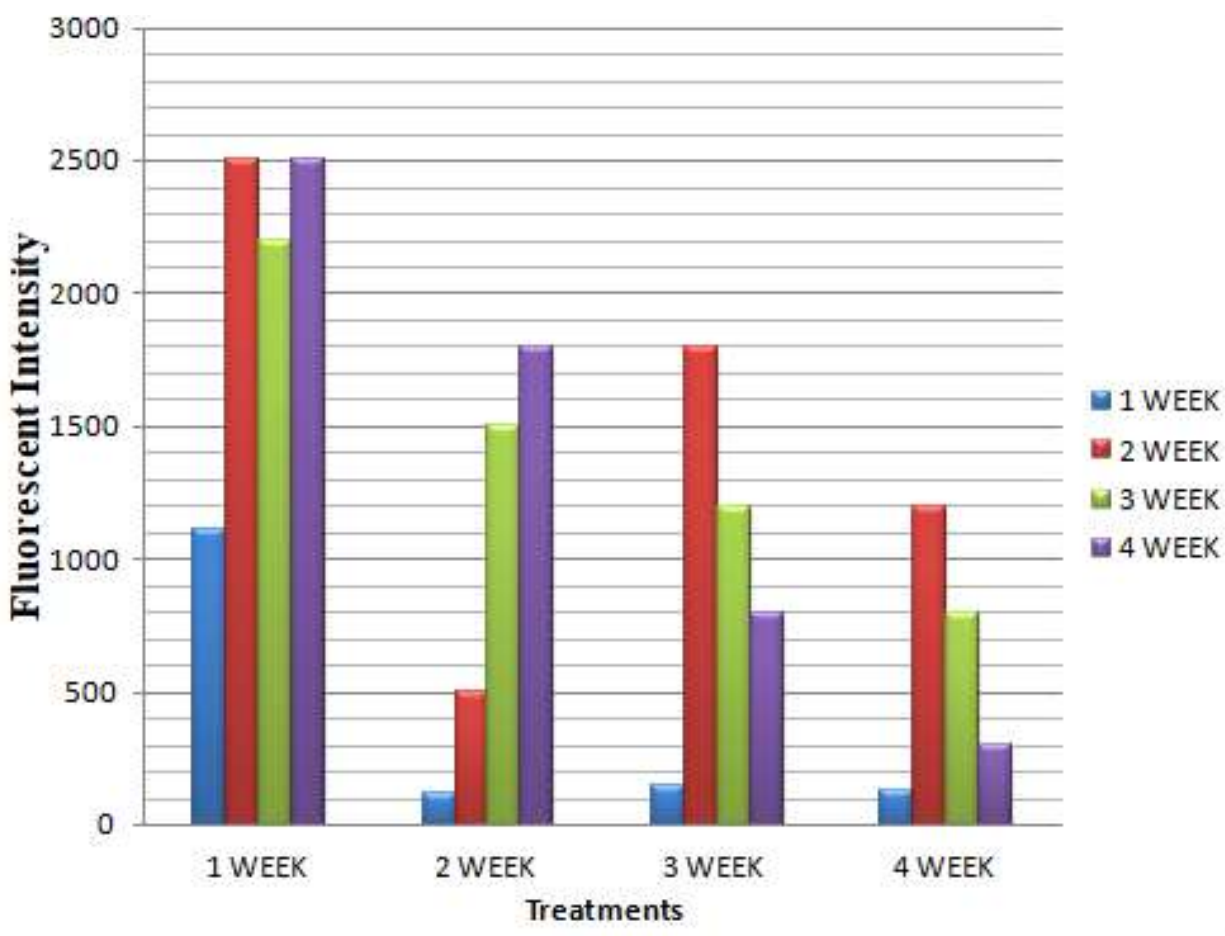


Fig: 2 Acute toxicity test

The zebrafish has been used as an experimental model due to its tiny size, inexpensive cost, and large clutch size^[39] as well as its striking physiologic similarities to humans. Because of this, employing this model could significantly advance pharmacological and toxicological studies. In the CSM toxicological test, there were no statistically significant differences between treatment groups in terms of survival rates ($p > 0.05$). The 24- and 48-hour survival rates in the 3,4-dichloroaniline group were 60% and 40%, respectively, while the rate in the CSM-treated group was

100% by the end of the study (96 hours). Based on the results, we have 99.9% confidence that the LC50 is greater than 100 mg/L [40].

Monitoring of blood glucose

To demonstrate the effectiveness of this approach, we monitored the blood sugar levels of zebrafish in three separate sets of tests. By checking the fish's blood sugar levels before and after meals, we were able to see how the four-day fast affected them. The time it took for zebrafish glucose levels to recover to normal was then measured using the insulin-potentiated glucose tolerance test (IP-GTT). After two days without food or liquids, sugar levels barely climbed before drastically declining on day three (Fig. 3). We discovered that three days of fasting was sufficient to get blood sugar levels back to normal. Despite the fact that there was no discernible difference in the mean between the 3- and 4-day fasts, the 4-day fast decreased the CV (Fig. 3A). The fish were fed again following a 4-day fast, and the results revealed that within 30 minutes of eating, glucose levels reach their high (Fig. 3B).

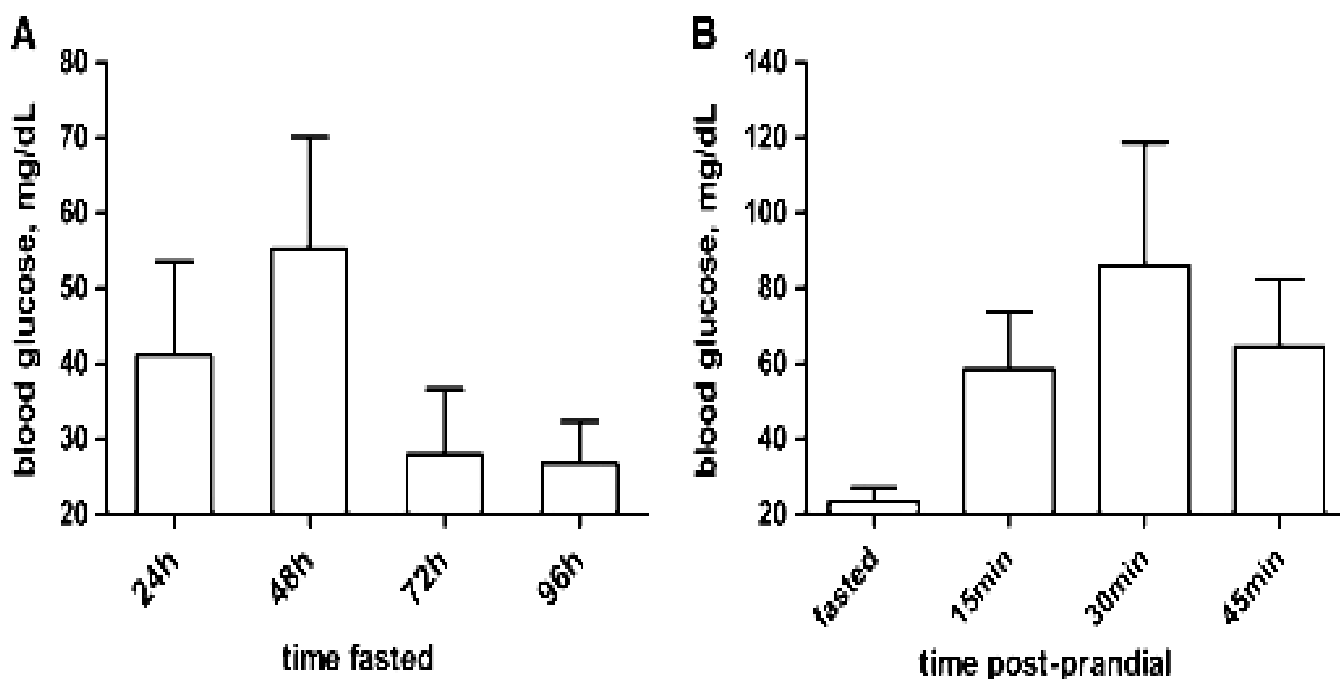


Figure 3: Fasting causes three distinct changes in blood sugar levels. Fasting blood glucose at 24 hours: CV = 30%, n = 35; at 96 hours: CV = 21%, n = 15; at 27%, n = 11; and at 31%, n = 15. The following was found by combining a Kruskal-Wallis test with a posttest based on Dunn's method: There was no discernible difference between the effects after 24 and 48 hours, 72 and 96 hours, or 24 and 72 hours. The data is presented with a mean and standard deviation. the concentration of glucose in the blood following a meal (B). After fasting for four days, the fish were fed once more. At least 10 fish should be sampled at each time interval. comparison of 30 minute glucose levels to 15 minute glucose levels (p 0.05); comparison of 45 minute glucose levels to 15 minute and 30 minute glucose levels (both p > 0.05); (p >0.01). After conducting a multi-way ANOVA, Tukey's posttest is performed. The data is presented with a mean and standard deviation. Both studies measured glucose concentrations using metre B.

Glucose inhibits haemostasis and accelerates diet-induced hyperlipidaemia in zebrafish larvae

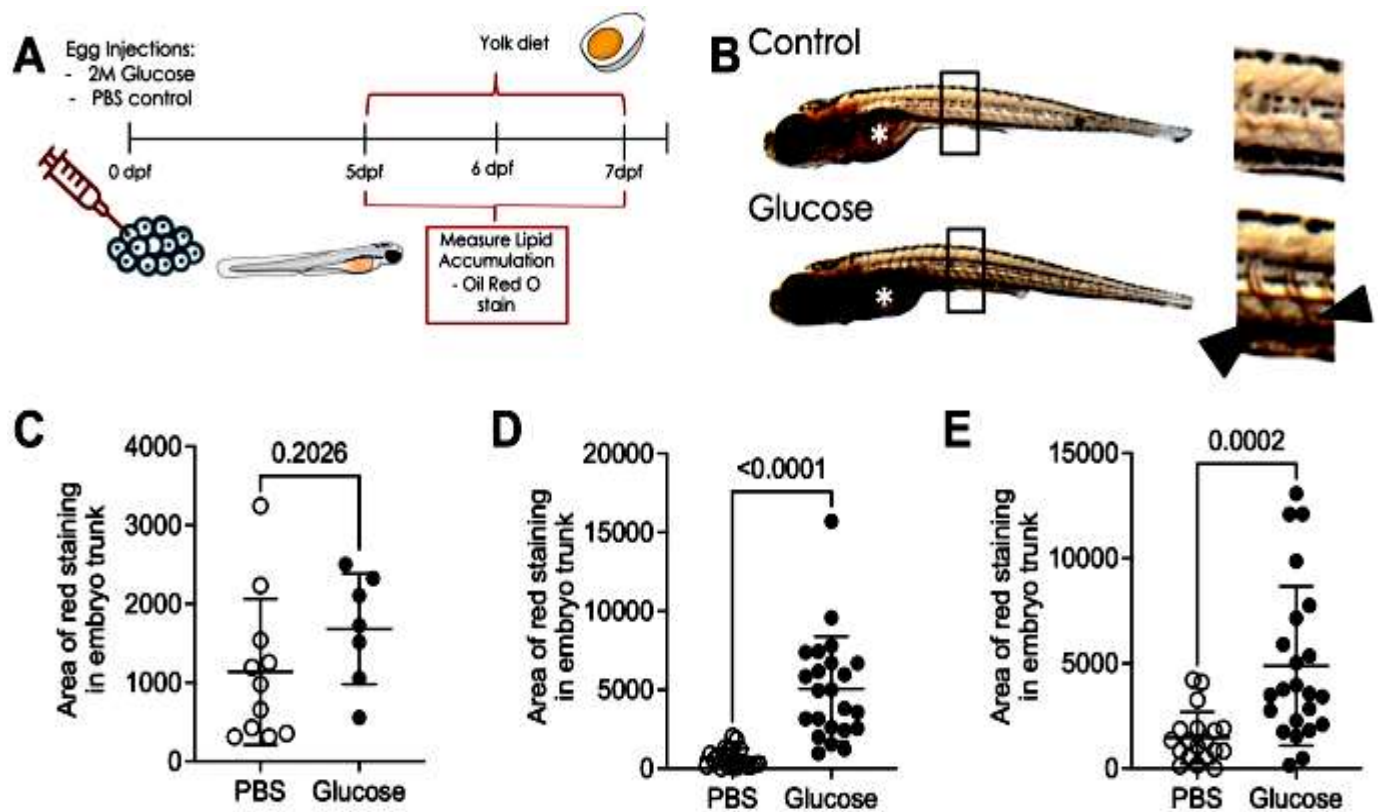


Fig: 4 Mammals have a close relationship between obesity and hyperlipidemia³³. We gave zebrafish larvae a high-fat diet of emulsified chicken egg yolk from five to seven days after fertilisation to examine if this connection is maintained in zebrafish (Fig. 4A). Five days after fertilisation, similar Oil Red O vascular staining was seen in larvae treated with glucose or PBS before being fed chicken egg yolks (Fig. 4B, C). Researchers discovered that 1 and 2 days after feeding, glucose-injected larvae had higher levels of vascular lipids (Fig. 4D,E).

Administration of Methanol Extract of *Vitex trifolia* L:

The extent to which CSM treatment suppressed AGE development is shown in Figure 5. After the trial period was over, all tested concentrations of CSM showed significant inhibition of AGE formation. CSM's greatest percentage of inhibition (96.5%, to be exact) was observed at a concentration of 90 mg/dL.

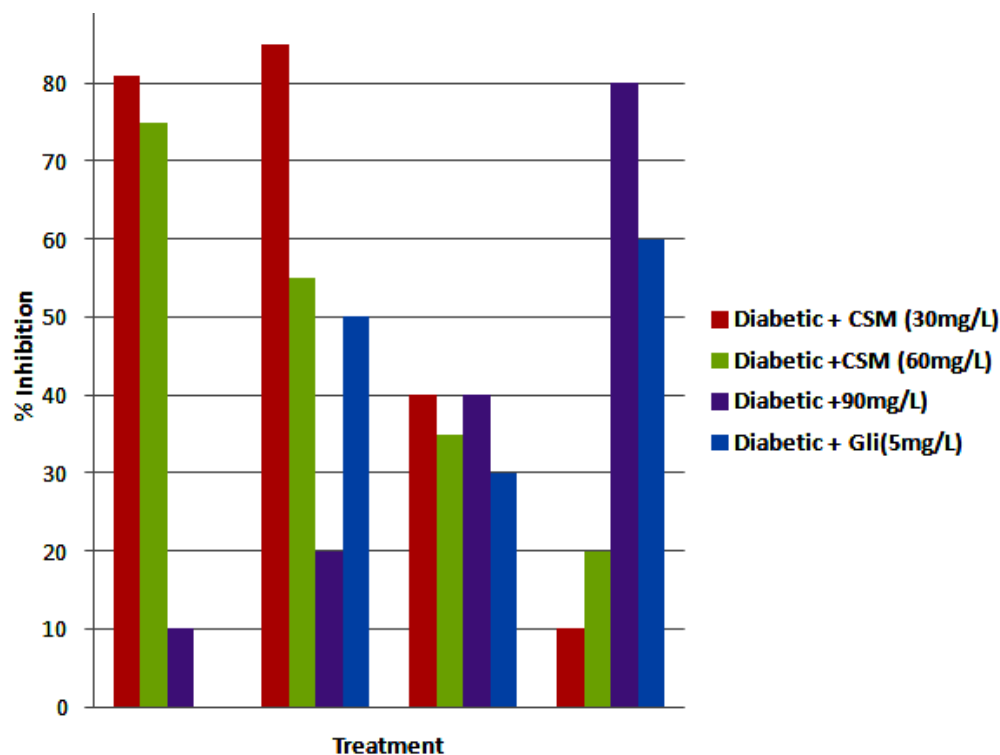


Fig: 5 Chronic exposure to CSM delays the onset of AGEs (14 days). Glycation inhibition percentages are displayed for each value. *Vitex trifolia L.* seed methanol extract is the active ingredient in CSM.

Conclusion

In persons with diabetes and obesity, the central nervous system might suffer from neuro-inflammation and oxidative stress when the blood-brain barrier (BBB) is damaged. Metabolic illnesses can cause abnormal behaviours in zebrafish and human models alike, as well as lessened brain plasticity. What physiological and molecular pathways underlying such aberrations, meanwhile, is still not entirely known. Here, we review the literature to demonstrate that zebrafish can be a valuable alternative model for examining the effects of metabolic disorders on brain homeostasis and NSC activity. Numerous research teams have developed effective zebrafish metabolic models to simulate obesity, diabetes, and elevated blood sugar. In fact, when given the right experimental conditions, zebrafish can display many characteristics of human metabolic disruption, such as increased body weight and BMI, expansion of visceral and subcutaneous adipose tissues, liver steatosis, disturbed lipidic profiles (LDL, HDL, body cholesterol, and triglycerides), insulin resistance, and hyperglycemia. Zebrafish is also a well-known model for studying a variety of features of brain plasticity, including homeostatic and regenerative neurogenesis. Therefore, this model can be applied to understand the impact of metabolic disorders on adult neurogenesis. The study of the molecular mechanisms and signalling pathways that are disrupted in NSCs under conditions of metabolic dysfunction is currently required.

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