



Pharmacological Investigation of Antioxidants and Antidiabetic Activity of Vitex Trifolia Bark In Diabetic Zebrafish Model

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Article History	Abstract
Received: 12 July 2023 Revised: 10 September 2023 Accepted: 27 October 2023	<i>Both diabetes and obesity have now been deemed global epidemics. Scientists coined "diabesity", to describe this relationship, as both obesity and diabetes are on the rise. Obesity increases the risk of heart disease, type 2 diabetes, cancer, and many other diseases. Both diabetes and obesity are complex illnesses that can be influenced both by hereditary factors and environmental ones. In order to develop pharmacological or surgical treatment for obesity and diabetes we need more research. It is therefore essential that animal models be used to better understand diabetes or obesity, and to develop effective treatments. Zebrafish are a good model for metabolic disease research due to their functional conservatism in terms of lipid metabolism and adipose tissue, pancreas structures, glucose homeostasis, etc. It is well suited to finding new ways of treating and preventing human diseases such as diabetes. The review examines both the benefits and drawbacks of using the zebrafish model for studying the diseases associated with diabetes and obesity. This review focuses on studies which have used zebrafish to model these diseases.</i>
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1. Introduction

Type 1 diabetes (DM) is one of the oldest and most persistent diseases known to mankind. The first mention of it was in an Egyptian text about 3000 years back. In 1936, researchers made the first distinction between type 1 diabetes and type 2. In 1988, diabetes of type 2 was recognized as an indicator of metabolic syndrome. Non-insulindependent DM (also known as type 2 diabetes) is the most common form of DM. Its hallmarks are hyperglycemia and insulin resistance. Due to increased risks of short-term as well as long-term complications in patients with diabetes type 2, they often die at an early age. Type 2 DM patients are at a higher risk of experiencing complications or dying, as the condition is common and advances slowly. It is also frequently misdiagnosed, especially in less developed regions such as Africa. In 2000, the WHO reported that India was home to 31 million diabetics (WHO). By 2030, the world population could reach 79 millions. Diabetes is treated by oral hypoglycemic medication, but these can have adverse side effects, such as haematological complications and changes in the organs' function, like liver and kidneys. There is no long-term treatment for diabetic nerve damage. But symptomatic treatment has not made any progress. Recent articles have covered the global search for medicinal plants that could cure diabetes. (Lakhtakia, 2020)

WHO suggests using herbal remedies as an effective and safe treatment for diabetes. Since thousands of years, people have relied on medicinal plants to treat a wide range of ailments. *Trifolium Vitex* is a deciduous bush known as *L. vari. Trifolia* is native to Asia, including China, India and Australia. *Vetiveria trifolia* has been used for centuries to treat inflammation. This plant is also known as *panikisanbhalu* in Hindi, *three-leaf Chaste Tree* (English), or *chaste trees* (Latin). Traditional Chinese medicine has used the dried fruits of *V.trifolia* (also known as *Fructus Viticis*) to successfully treat various ailments. These include rhinitis and common cold, headaches, impaired vision, ocular swelling, and ciguatera fish poisoning. The seeds of the *V. Trifolia* are used in traditional medicine to treat fish poisoning and inflammation. (Kooti, 2020)

According to some sources, the roots are antiemetic, expectorant and fever-reducing. According to some sources, roots of *V. trifolia* are antiemetic and expectorant. Analysis of seeds extracts revealed flavonoids like casticin and vitexin as well as terpenes such as eucalyptol. Researchers have confirmed the anti-inflammatory effects of *V. Trifolia* leaf extractions by using mouse and rat models (e.g. RAW264.7 cell lines generated from lipopolysaccharide-induced paw-*edoema* rats [18]). It is not clear what the inflammatory effect of *V.trifolia* can have on macrophages in humans, or the solvents available to extract its active components. The inflammatory stimuli *phorbol 12 myristate 13 acetate* can be used on human monocytic cell lines such as U937 to help them develop into macrophages. This can then be used for studying macrophage function. *Trifolia* is one of Singapore's most popular fresh herbs, and we have described it in a previous ethnobotanical research. The leaf extracts of the *trifolia* plant inhibit the growth of cancer cells. (Wee, 2020)

2. Literature Review

The metabolic disorder *diabetes mellitus*, characterized by high blood sugar levels and hyperglycemia is often linked to oxidative damage or impaired antioxidant defences. Natural products and plant-derived compounds have gained attention in recent years for their potential to manage diabetes and its associated complications. This review of literature focuses on the pharmacological evaluation of antioxidants in *Vitex Trifolia* Bark using a

diabetic Zebrafish Model. This review explores current research and highlights the benefits of this natural remedy.(Unuofin, 2020)

The Antioxidants in Diabetes

The hallmark of diabetes is oxidative stress, which results from an imbalance in the production and defenses of the antioxidants. Increased ROS can cause cellular damage, and may contribute to diabetes complications. Antioxidants have been shown to be effective in reducing oxidative stresses and their adverse effects.(Matough, The hallmark of diabetes is oxidative stress, which results from an imbalance in the production and defenses of the antioxidants. Increased ROS can cause cellular damage, and may contribute to diabetes complications. Antioxidants have been shown to be eff, 2022)

Vitex Trifolia - A Source of Natural Antioxidants:

Vitex trifolia is also known as "Indian Privet", "Nirgundi" and has been used in Ayurvedic medicine for centuries. The bark of the plant has been studied for its potential pharmacological effects. Recently, studies focused on antioxidants such as flavonoids and phenolic compounds in Vitex Trifolia Bark.(Ahuja, 2021)

Anti-Diabetic Activity in Vitex Trifolia bark:

Researchers have found that Vitex trifolia extracts could be anti-diabetic. Potential mechanisms of action may include:

Improvement in Insulin Sensitivity - Some studies have suggested that the bioactive components found in Vitex trifolia may increase insulin sensitivity. This would allow glucose to be taken up by cells, and blood sugar levels reduced.(Ukiya, 2020)

Protecting Beta-Cells:

The bark extracts have been shown to protect beta cells in the pancreas against oxidative damage. This preserves their insulin secretion ability and function.

Antioxidant Effects:

Vitex trifolia is believed to contain antioxidants that fight oxidative stresses, reduce the risk of complications related to diabetes and improve metabolic health.

Zebrafish Model for Diabetes Research:

Zebrafish have become a popular model for research on diabetes due to their genetic similarities to humans, and transparency. This allows for non-invasive monitoring physiological processes. Diabetic Zebrafish Models provide an excellent platform to investigate the anti-diabetic properties of natural compounds.(Sharchil, Zebrafish have become a popular model for research on diabetes due to their genetic similarities to humans, and transparency. This allows for non-invasive monitoring physiological processes. Diabetic Zebrafish Models provide an excellent platform to inves, 2022)

Future directions and Current Research:

Recently, studies using diabetic zebrafish as models explored the effects on Vitex trifolia extracts of glucose metabolism, insulin resistance, and markers for oxidative stress. Vitex trifolia may have potential as an adjunctive treatment for diabetes. Further research is required to determine the bioactive components responsible for these effects, and their mechanism of action.(Dandin, 2022)

Vitex trifolia, a traditional remedy, has been studied pharmacologically to determine its antioxidants and anti-diabetic properties. Further exploration is needed to determine the potential benefits that this traditional remedy can offer, especially in reducing oxidative stresses and improving glucose metabolism. Research in this field may eventually lead to novel therapies for diabetic complications and diabetes.

The glucose transporter GLUT is found in the gills of zebrafish (GLUT 1, 3, 6, 8, 10, 13) and in their intestines (GLUT 5, 9). As reported by Gleeson and colleagues in 2007, our results indicate that exposure to glucose solutions of 111 mM promotes an increase in blood sugar levels in zebrafish. This, in turn, leads to the glycosylation in their eyes. CSM counteracts hyperglycemia by increasing insulin production, according to our results. It has also been suggested that natural products have a greater effect on high blood sugar levels due to the antioxidant properties of these products. CSM's ability to lower blood sugar will require further studies. (Ahmed, 2023)

The mitochondrial fission/fusion process is closely related to the mitochondrial function. It can also influence mitoROS and vice versa. van der Bliet and Wai and Langer provide a comprehensive overview of mitochondrial fusion and fission. In the case of mitochondrial fission (dynamin related protein 1), small cytoplasmic GTPase is Drp1. In a wide range of environmental conditions, Drp1 regulates mitochondrial function and mitoROS. Oxidative stress affects Drp1 mitochondrial fission and mitochondrial translocation. Oxidative stress can regulate mitochondrial fission, and it is also thought that mitophagy acts as a negative regulator for mitoROS signals through the selective degradation of malfunctioning mitochondria. Data suggest ROS signals are responsible for serine 616 activation by Drp1 and its post-translational modification, including S-nitrosylation. The contribution of ROS to other fission protein regulation is unknown at this time and is ripe for further investigation. (Forrester, 2022)

Autophagy, a catabolic reaction that is highly conserved in nature, involves the delivery of cytoplasmic organelles and macromolecules to lysosomes. Autophagy at its constitutive levels supports metabolic homeostasis, promoting an equilibrium between organelle biogenesis, degradation and protein synthesis. Bento et al. provide a comprehensive review of the regulation and autophagic mechanisms.

Material and methods

Searching from multiple sources: Web of Science, Scopus NCBI Google Scholar, Key words: Zebra Fish, Plant, Hyper Lipidemic, and Anti Diabetic Activity:

- Plant Extraction
- Collecting Plant Material
- Plant Extract Preparation

Cold maceration was used to make the aqueous extraction. 100 grammes dry powder, which is coarse and not fine, was combined with 100 millilitres water. It was macerated at room temperature for 18 hours. Place on a plate and let dry in an evaporating pan.

Phytochemical screening at preliminary level

An aqueous extract from V. trifolia seed was examined for its phytochemical composition. It revealed that the seeds contained steroid, sugars and amino acids.

In animals and vitro glycation BSA

In this study, primary adult zebrafish that were bred solely for reproduction was used. In a typical breeding tank, three adult male zebrafish mature and one wild-type female zebrafish would be present. To ensure that the timing of the progress is correct, we separated the sexes using a solid barrier. Construction didn't start until the next morning. After removing the divider, we strained the embryos out of the tank using a petri dish with a diameter of 60mm. Each dish contained less than fifty embryos. At this stage, embryos consist of a single cell. The embryos under the microscope are examined repeatedly to make sure they're healthy and viable.(Hoo, 2021)

In vivo fish model

The toxicity test was carried out in compliance with OECD standards. The addition of eight fish in batches to five-litre tanks with water at 24 \pm 2 degrees Celsius and continuous aeration was performed. 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). Every 24 hours, CSM and 3,4-dichloroaniline extracts were swapped to maintain the concentration. The various fish batches were not allowed to eat for 96 hours while the conditions above continued. The fish were regularly examined throughout the development of the test to determine their swimming patterns and gill activities.(Fish, 2022)

Hyperglycemia is thought to be responsible for a number of clinical features in diabetes. The characteristics of diabetes in mammals include changes to the leukocyte's biology, changes in hemostasis and an increasing number macrophages with a high lipid content. Studies on mammals suggest that hyperglycemia-induced microvascular damage lowers the production of endothelium adhesion molecules necessary for immune cell recruitment. In turn, diabetic lesions do not draw neutrophils or macrophages nearly as often. The inflammatory environment of diabetic lesions causes macrophages to adopt an inflammatory phenotype.(Rask-Madsen, 2019)

3. Results and Discussion

The results of an initial phytochemical study of the seeds revealed a wide range of phytoconstituents including carbohydrates, phenolics compounds, flavonoids and amino acids.

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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	+	-	+

In vitro glycation BSA

Fluorescence levels above normal are an indication of ageing. The fluorescence of the BSA/glucose systems is shown in Figure 2. This shows how it changes after one, two, three and four weeks. As BSA, CSM, glucose and CSM concentrations rise, the fluorescence of incubated samples decreases. The concentration of 5mg/mL was the most affected (90.45%), compared with glycated BSA, after 4 weeks incubation. BSA/glucose/AG showed a decrease of 91.8%.

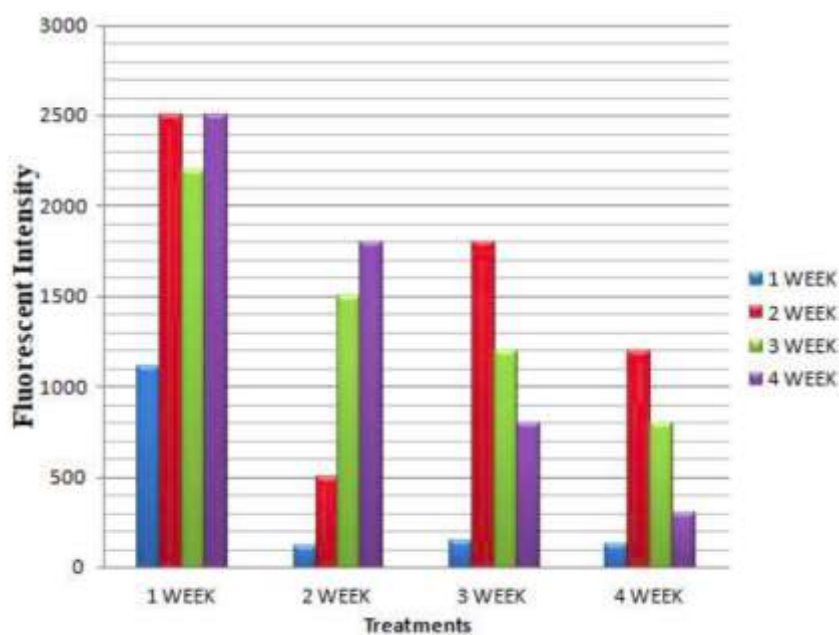


Figure: Acute toxicity test

Zebrafish is used in experiments because of its small size, low cost and large clutch [39], as well as the striking similarities between its physiology and that of humans. This model can be used to advance toxicological and pharmacological research. There were no statistically significant survival differences in the CSM test ($p < 0.05$). In the group treated with 3,4-dichloroaniline, the survival rate was 60% for 24 hours and 40% after 48 hours, whereas the CSM group had a 100% success rate at the conclusion of the study. We can be 99.9% confident that the LC50 value is higher than 100mg/L based on our results.

Monitoring of blood glucose

In order to demonstrate this method's effectiveness, we tested the blood sugar of three different zebrafish. We were able to observe the effects of the fast on the fish by checking their blood sugar before and after each meal. The insulin-potentiated gluco tolerance test (IP GTT) was used to measure the time taken for glucose levels in zebrafish to return to normal. Sugar levels barely increased after two days of fasting. They then dropped dramatically on the third day. Three days fasting is enough to bring blood sugar back to normal. The CV decreased despite the fact there was no difference between the three- and four-day fasts. After a four-day fast the fish were again fed. The results showed that glucose levels reached their highest within 30 minutes after eating. (Eames, 2021)

Conclusion

When the BBB is compromised in people with obesity and diabetes, they may experience neuro-inflammation as well as oxidative stresses. In both zebrafish models and in humans, metabolic illnesses may cause altered behaviours as well as a reduced brain plasticity. It is not yet clear what physiological or molecular mechanisms are responsible for such abnormalities. We review the literature in order to show that zebrafish is a useful alternative model to examine the effects of metabolic diseases on brain homeostasis. Numerous research teams have created effective zebrafish models to simulate diabetes, obesity and high blood sugar. When given the correct experimental conditions, Zebrafish are able to display many of the same characteristics as humans, including increased weight, BMI, visceral and under-skin

adipose tissue, liver steatosis and altered lipid profiles (LDL and HDL), hyperglycemia, insulin resistance and dyslipidemia. Zebrafish are also well known as a model to study a range of aspects of brain plasticity including homeostatic neurogenesis and regenerative neurons. This model is therefore useful for understanding the effects of metabolic disorders in adult neurogenesis. Currently, it is necessary to study the signalling and molecular pathways disrupted by metabolic disorders in NSCs.

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