RESEARCH ARTICLE

Effect of methanolic extract of *Dendrophthoe falcata* leaves on acute hyperglycemic adult zebrafish

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Received: January 18, 2022; Accepted: February 14, 2022

ABSTRACT

**Background:** Herbs used in medicinal practices by the indigenous healers are found to be of great importance in the management of diseases that are yet to have a cure by the available drugs. Practice of using herbs available in the vicinity by the locals as medication for ailments is a universal phenomenon. *Dendrophthoe falcata*, an arboreal parasitic plant used in the indigenous medicine for the management of diabetes, is explored here for its acute hyperglycemic model adult zebrafish. **Aim and Objectives:** The aim of the study was to determine the hypoglycemic effect of the methanolic extract of *Dendrophthoe falcata* leaves in alloxan-induced acute diabetic adult zebrafish. **Materials and Methods:** Adult zebrafishes were grouped into five groups with six fishes in each group exposing them to alloxan to induce acute hyperglycemia and then treating them with two test doses of 40 mg/dl and 60 mg/dl of the methanolic extract of the plant extract. Another group was treated with metformin with a dosage of 20 micro moles. Body mass index, blood glucose, and histopathological examination pre- and post-treatment for a period of 14 days were studied. **Results:** The effect of the herbal extract in both the doses was promising when compared with the standard drug metformin; however, the cytoprotective effect was very predominant with the both doses of the extract. Acute hypoglycemic was comparatively good when compared with the standard group treated with metformin. **Conclusion:** The antidiabetic effect of the arboreal parasitic plant has been established with a need for further exploration of this plant for a potential drug for diabetes mellitus.

KEY WORDS: Diabetes Mellitus; *Dendrophthoe falcata*; Hypoglycemic Effect; Medicinal Herbs; Cytoprotective; Arboreal Parasite Plant

INTRODUCTION

India stands next to China in having people with diabetes mellitus. More than 69.2 million people were living with this metabolic disorder.[¹] The disease is of two types: Type I and Type II diabetes. Type II diabetes is usually explored with various animal models, in which, the beta cells of the endocrine pancreas are damaged with either alloxan or streptozotocin, resulting in induced hyperglycemia.[²,³] As the cellular processes involved in the synthesis of proteins are conserved in the zebrafish, these animals are recently used in wide range of research associated with genetic disorders and acquired diseases.[⁴] Due its small size, cost-effectiveness in its maintenance, and also due to the transparent embryos they produce, they stand-out as an animal model for diabetes research.[⁵] As they have genomic similarity...
to that of humans, they are also used for studying the functions of human genome.[4]

Dendrophthoe falcata, an arboreal parasitic plant, has been established with medicinal properties which is evident from its wound healing, anti-microbial, antioxidant, and anti-nociceptive properties.[6,7] Inhibition of carbohydrate hydrolyzing enzymes such as α-amylase activity, α-glucosidase, β-glucosidase, and sucrase activity of the D. falcata is established in vitro.[8]

The fear of side-effects by the synthetic drugs and the contraindications during which it cannot be administered to people has lured them to avoid the drugs.9,10 Low cost, more potent, and yet, with less or no side effect drugs are the basis for new drug discovery across the globe. In this study, we aim to understand the hypoglycemic effect of D. falcata in zebrafish models. Here, in this study, we have explored the hypoglycemic effect of the methanolic extract of D. falcata leaves in alloxan-induced acute diabetic adult zebrafish.

MATERIALS AND METHODS

Institutional Animal Ethics Committee clearance was obtained before the initiation of the study (IAEC/SSSMCRI/2020-10/ dated February 14, 2020). The project was done for a period of 2 months post-COVID lockdown in this part of the country.

Collection of Plant Material and Authentication

D. falcata on host plant Mangifera indica was collected from Kaliakkavilai (Kanyakumari District) and was authenticated by a plant taxonomist. Plant samples were deposited with the institute with which the taxonomist was working for the future references and repository purposes.

Preparation of Plant Extract

Shredded plant leaves were completely shade dried for a period of 20 days and were then powdered and extracted with methanol using soxhlet apparatus and later concentrated in a rotary vacuum evaporator.

Acute Toxicity Testing of D. falcata Leaf Extract

Guidelines on acute toxicity (LC50) by the Organisation for Economic Cooperation and Development (OECD-203) guidelines for testing of chemicals were followed.[11] Ninety-six h acute toxicity experiment was done on adult zebrafish with an average body weight of 250 g and length of 2.6 cms.

Zebrafishes were grouped into seven separate water tanks and were randomly selected with both male and female fishes. All of them were exposed five different concentrations of the methanolic extract of D. falcata (20, 40, 60, 80, and 100 mg/ml). Mortality was determined continuously and the dead fishes were confirmed with there is loss of opercular movement. Fishes were disturbed using a glass rod to confirm the mortality. Fishes were not fed before or during the experimental observation. Behavior of the fishes was also monitored regularly and all the relevant details were documented.

Zebrafish Maintenance and Groups

Adult zebrafishes (wild strain) with a bodyweight of about 200–300 mgs and of both sexes were purchased from the local breeders and housed in a stock tank in our departmental zebrafish laboratory for a period of 15 days for acclimatization. Alternate light and dark cycle of 14 h and 10 h was maintained to provide them with the natural yet controlled light cycle and thereby the circadian cycle. These fishes were later grouped into five groups as below,

- Group I (10): Normally fed controls
- Group II (10): Alloxan-induced diabetic controls (negative) (300 mg/dl)4
- Group III (10): Alloxan-induced diabetes plus methanolic extract of D. falcata (40 mg/dl)
- Group IV (10): Alloxan-induced diabetes plus methanolic extract of D. falcata (60 mg/dl)
- Group V (10): Alloxan-induced diabetes plus drug (metformin) (20 μM).[12]

Fishes were maintained as per the standard procedures described by Zhang et al. 2017.[13] Water in the tank was filtered of the dirt by the continuous overflow hang on filters, and all the necessary parameters such as pH and TDS were kept in control with the quality care guidelines given by the CPCSEA, Government of India guidelines on zebrafish.

On the initial day of the experiment, Group II, III, IV, and V fishes were experimented as per the following protocol.[13]

- Phase I: Thirty min exposure individually with the 300 mg/dl alloxan monohydrate in half normal saline water
- Phase II: Thirty min exposure individually with the 30 mg/dl of 1% D glucose
- Phase III: Sixty min exposure individually with the ordinary tank water
- Phase IV: Oral dosing (5 μL) of the Group III fishes with 40 mg/dl of the methanolic extract and Group IV fishes with 60 mg/dl of the methanolic extract for 7 days
- Phase V: Exposure of the hyperglycemic fishes with 20 μM of Metformin for 7 days.

After 7 days of exposure of the drug, the zebrafishes were euthanized using ice cold water and the different parameters such as body weight, length of the fish, blood glucose, and histopathological sections of viscera were done.

Body Mass Index (BMI) Determination

Length of the fishes was determined after ice cold anesthesia and then the fishes were taken to lie on a petri dish which has
a standard calibrated graph paper. Length was measured in centimeters from the tip of the snout to the caudal end of the body. Photos were taken with respective labels and the length was calculated later from the image using a computer. Body weight was measured by transferring the fishes into a small container with known quantity of fluid for very transient time period and with the use of the tare option in the standard weighing machine. BMI of the fishes was determined using the formula body weight (g) divided by the square of the fish body length (cm).\textsuperscript{14}

**Blood Glucose Determination**

We have used a 36 G needle which was already soaked in heparin to puncture the dorsal artery on the spot identified between the caudal and the anal fin. The bleeding was continued till a good droplet is formed and blood glucose was estimated using ACCU-CHEK Instant S blood glucose estimating device.

**Histopathological Changes**

Histopathological analysis on the islets of Langerhans of the zebrafishes of the various groups was performed to observe the extent of damage that is an indication of the acute hyperglycemic model induced through alloxan. Histopathological slides in this study were prepared using Hematoxylin and Eosin staining.\textsuperscript{13} Formalin (10%) preserved fishes were processed with increasing grades of isopropyl alcohol for dehydration, cleared with xylene, and impregnated with paraffin using automatic tissue processer (Yorko, India). Further these fishes were embedded with paraffin using an automatic tissue embedder (Yorko, India). Then, the paraffin blocks obtained were removed from molds and the stored at room temperature until sectioning. Each individual fish was serially sectioned on a rotary microtome (Leica) with 5 \( \mu \)m thickness as the setting. Later, theses slides were deparaffinized with hot plate before staining. Hematoxylin and Eosin staining was done and slides were mounted with DPX mounting media. Representative images were taken using Adelta digital microscope and saved for further image analysis.

**Statistical Analysis**

The data were expressed as means ± standard error of the mean (SEM). Statistical comparisons were performed using the Kruskal–Wallis test for non-parametric data. \( P < 0.05 \) was considered to be statistically significant.

**RESULTS**

**Acute Toxicity Study**

As per the OECD-203 guidelines on lethal toxicity, estimation on the dosage of lethality was determined and was found to be >100 mg/dL. Zebrafishes were grouped in such a way that there is no disparity in the body weight or BMI among the group members of the zebrafishes. There was no mortality in any of the concentrations that we took ranging from 20 mg/dl to 100 mg/dl. Hence, the LC\textsubscript{50} concentration was beyond 100 mg/dl and our test dose concentrations were not lethal to the fishes. The BMIs among the fishes in the group were of equivalent to each other with no statistically significant differences which is explained in the Figure 1.

Alloxan was the choice of drug for the induction of acute hyperglycemia in the groups and was very significantly established by the increased blood glucose level of about 468 mg/dl to that of the 49.75 mg/dl of the control group fishes. These findings were of statistical significance as depicted in Figure 2 that establishes the acute hyperglycemia set by the drug alloxan.

After the study period, the fishes in different groups differed in their weight and length; yet, there were no statistically significant changes which showed the intervened herbal extract showing no changes in the bodyweight and length, that is, BMI. Figure 3 exhibits the non-significant BMI changes noted in the groups after the experimental study period.

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![Figure 1: BMI of the zebrafishes (mean ± SEM)](image1)

**Figure 1:** Body mass index (BMI) estimation showing that the groups are of equivalent BMI in which the mean BMI of the fishes among the groups was similar (\( P > 0.05 \): Non-significant)

![Figure 2: Blood glucose level after the administration of alloxan (mean ± SEM)](image2)

**Figure 2:** Blood glucose estimation in the alloxan-induced hyperglycemic group (*\( P < 0.001 \): Very significant)
After the study period, the changes in the blood glucose level which were initially increased as a result of acute hyperglycemic induction were completely stabilized by the two different doses of the methanolic extract of the herb *D. falcata*. Furthermore, Figure 4 has showed the efficiency of herbal doses as equivalent to that of the standard drug of Metformin to bring about a hypoglycemic status with the acute-induced hyperglycemic zebrafishes. Figure 4 explains the comparative hypoglycemic effects in the different groups against the standard drug.

**Histopathological Changes**

The changes in histological architecture of the endocrine pancreatic tissue in Hematoxylin and Eosin staining show the changes that occurred after the study period with alloxan and extract treated groups in the following figures explaining the cytoprotective nature of the *D. falcata* extract [Figures 5-9].

The integrity of the endocrine as well as the exocrine cells was maintained with the extract in the treatment groups and is comparatively equivalent to the effect of the standard drug Metformin.

**DISCUSSIONS**

*D. falcata* is an arboreal parasitic plant with a number of medicinal benefits based on the host plant it grows with. The presence of various phytochemicals such as flavonoids, terpenoids, and alkaloids has been found to be an important source of the medicinal property that the plants have exhibited. They were found to be of various medicinal properties such as in improving cognitive functions, treatment of impotence, paralysis, as aphrodisiac, astringent, narcotic, diuretic, asthma, wounds, ulcer, and for pulmonary tuberculosis.[16] Other problems such as skin diseases, menstrual problems, fractures, hepatoprotective, and anti-tumor activities.[16]

Our study results were convincing to claim the plants extract to have a potential hypoglycemic and hepatoprotective effect. We also have some of the research findings that have showed the antidiabetic effect of the plants leaf extracts. They differ in their findings only by the plants host plant, especially all
these findings are from the *Azadiracta indica*, whereas in this study, the host tree was *Mangifera indica*.\(^{[17]}\)

Hypoglycemic effect of the methanolic extract of *D. falcata* has not been explored much. As per the literature available, quercetin a flavonoid content in the leaf extracts of *D. falcata* which are primarily antioxidant in nature, that reduces inflammation, and the important ameliorating efficiency on hyperglycemia and dyslipidemic could be the possible effects by which the hypoglycemic effect was established in this study.\(^{[2]}\)

Antidiabetic activity of the extract of *D. falcata* has been well established in alloxan-induced diabetic rats.\(^{[18]}\)

The methanolic extract of *D. falcata* leaves (host tree *Azadirachta indica*) were found in another scientific study to be of more efficient in bringing about the antidiabetic effect by the four different enzyme assays done such as α-amylase, α-glucosidase, β-glucosidase, and surcase activity. By these activities, they have suggested the herb *D. falcata* as a potent herb to manage the hyperglycemic effect in diabetes mellitus.\(^{[8]}\)

The cytoprotective effect of the methanolic extract of *D. falcata* has been documented in another study with alloxan induced diabetic rats at a dosage 200 mg/kg. It has protected from the cellular damages of alloxan by the extract. The effects are anti-hyperglycemic activity by reduction in the blood glucose level and improvement in the bodyweight.\(^{[19]}\)

Our study results with the methanolic extract of the plant have shown an equivalent hypoglycemic effect at both the doses (40 mg/dl and 60 mg/dl) to that of the standard Metformin drug. Our study in zebrafish animal models was to be the first of its kind to establish the hypoglycemic effect of this particular *D. falcata*.

**CONCLUSION**

The results of this study have substantiated the antidiabetic use of the arboreal parasitic plant *D. falcata* on the host tree *Mangifera indica* by the indigenous healers for the management of hyperglycemia in diabetes mellitus. Furthermore, investigations have to be carried out on isolated phytocompounds of this plant in the treatment of diabetes mellitus.

**REFERENCES**

2. Gohil T, Pathak N, Jivani N, Devmurari V, Patel J. Treatment with extracts of Eugenia jambolana seed and *Aegle marmelos*


Source of Support: Nil, Conflicts of Interest: None declared.