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# Hepatoprotective effect of methanolic extract of *Dendrophthoe falcata* leaves in paracetamol induced liver toxicity in adult Zebrafish (Danio rerio)

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**Abstract**

Liver diseases being one of the most widespread medical illness and paracetamol being an over to counter drug, demands for an increased effort when it comes to treatment and relevant research. This study aims to assess the hepatoprotective effects of a methanolic extract of *Dendrophthoe falcata* leaves on Paracetamol-induced liver toxicity in adult Zebrafish (*Danio rerio*). The experimental adult zebrafish were exposed to 5mM paracetamol to induce liver toxicity which was followed by therapeutic treatment with methanolic extract of *Dendrophthoe falcata* leaves with two different concentrations of 40mg/l and 60mg/l that were separately administered to two different groups of experimental fishes. Besides the test drug groups, one unexposed control group one paracetamol control group and a group delivering the conventional drug silymarin also were added for better comparative analysis. Histopathological reports revealed the extent of liver damage through analysis of the condition of hepatocytes, portal triad structures, central vein and sinusoids. The results indicated that the hepatocytes and other clinical parameters were found normal among the zebrafishes treated with the test drug as well as silymarin whereas the Paracetamol control portrayed mild degeneration in the periportal hepatocytes, dilated sinusoids, Congested central vein and periportal inflammation. Thus, this study concludes that methanolic extract of *Dendrophthoe falcata* leaves is equally potent to that of the conventional drug silymarin in treating paracetamol induced liver toxicity.

**Keywords**: *Dendropthoe falcata*, hepatoprotective, Zebrafish disease model, Paracetamol induced liver damage, Stigmasterol.

## 1.Introduction

Liver diseases are one of the fast-emerging health problems, globally [1]. In India, liver diseases are rapidly being recognized as public health concerns. Of the two million liver disease-related deaths worldwide in 2015, 18.3% occurred in India due to a widespread epidemic of liver disease[2]. India's shift in culture and habits to a western diet, sedentary lifestyle, and societal acceptance of alcohol have led to a rise in liver diseases [3]. Progressive sedentary lifestyle, consumption of diet dense in calories, spiralling upward trend in alcohol consumption, early age of alcohol consumption, and smoking has been identified for the increased burden on liver disease in our country. There exists a high prevalence of co-existing risk factors such as obesity and diabetes [4,5].

Non-alcoholic, alcoholic and the count of drug induced liver diseases are persistently increasing in our country and with this changing face of burden of liver diseases [4], it is the right time to explore for new drugs that can be cheaper and indigenous. Due to the use of alcohol, many disorders including Over 25% of alcohol-related deaths globally are attributable to liver disease[3]. Paracetamol, commonly used to treat fever and pain, is safe at the therapeutic dose of 4 g/day. However, it has consumption-dependent hepatotoxicity and can cause serious liver impairment with a single dose of 10-15 g [6]. Damage to the liver due to such effects are an increasing medical, scientific, and public health concern. There are not many alternatives to therapy for common liver injuries, and current medications may be less effective [3].

Exploring the knowledge of our indigenous medicinal practices and scientific validation of the herbs used by our people in traditional medicines will help us identify more potential drugs to treat the diseased population in our country. Based on these we are proposing this study to explore the hepatoprotective nature of the herbs being used by the locals in their traditional medicinal practice. We have identified the herb *Dendrophthoe falcata* used by the local population (Kanniyakumari District) in traditional medicine for treating liver diseases which might have the potential to be a hepatoprotective agent /medicine. *Dendrophthoe falcata* have traditionally been utilized in Indian and Indonesian ethnomedicinal platforms for treating a variety of conditions, including cancer, ulcers, asthma, paralysis, skin diseases, tuberculosis, and menstrual problems [7].

Zebrafish (Danio rerio) are commonly employed as animal models due to their quick generation period, high fertility, and inexpensive housing and maintenance costs[8]. As a result, zebrafish have grown in popularity as an animal model for toxicological research [8,9]. Hence, we plan to explore the potential of *Dendrophthoe falcata* using adult zebrafish as the disease model. *Dendrophthoe falcata,* an arboreal parasitic plant, has been shown to have medical qualities such as wound healing, anti-microbial, antioxidant, and antinociceptive [7]. *Dendrophthoe falcata*, an imperil plant found in the Kanniyakumari district, is frequently employed by traditional healers to treat liver diseases alongside other herbs. It has been scientifically validated for its great antioxidant properties, which are analogous to conventional treatments [7].

Methanolic extract of the leaves has shown a higher antioxidant activity and a larger quantity of the presence of Quercitrin as a major phytochemical for its property [10,11]. The hepatoprotective properties of Dendrophthoe falcata leaf methanolic extract will be investigated here. The phytochemical investigations priorly performed revealed high levels of terpenoids, flavonoids, tannins, and modest amounts of phenols and glycosides[12,13]. After conducting immense research Silymarin is proven to treat liver damage in hepatic diseases [14].

Therefore, Silymarin is currently used as the standard drug for hepatotoxicity. In this work, the hepatoprotective effects of a methanolic extract of Dendrophthoe falcata leaves on paracetamol-induced liver injury in adult zebrafish are investigated, considering the standards of the extant literature.

## 2.Materials and Methods

## 2.1 Animals

Adult zebra fishes (wild strain) were purchased from the local breeders and were housed under a stock tank in our departmental zebrafish laboratory for a period of fifteen days before the Experiment. Alternate light and dark cycle of 14 hours and 10 hours were maintained to provide them with the natural yet controlled light cycle. The study was approved by the Institutional Animal Ethics Committee before to its commencement, and all protocols pertaining to zebrafishes were conducted in accordance with the recommendations given by the Animal Welfare Board and CPCSEA, the Government of India.

## 2.2 Plants

*Dendrophthoe falcata* on host plant *Mangifera indica* was collected from kaliakkavilai (Kanniyakumari District) and it was authenticated by a taxonomist.(Dr. K. Jeyaprakash, Botanist, Institute of Natural Science Research, Devadanapatti-625602)

## 2.3 Preliminary phytochemical screening

The *Dendrophthoe falcata* leaves were subjected to phytochemical screening to verify the presence or absence of various metabolites tested with methanol and water extract (Table-1)

**Table 1 - Phyto Chemical Analysis – Leaf Methanol Extract.**

| TEST | METHANOL | WATER |
|---|---|---|
| **Alkaloids** | + | + |
| **Flavonoids** | + | + |
| **Glycosides** | + | - |
| **Steroids** | - | - |
| **Protein** | - | - |
| **Tannins and Phenolic compounds** | - | - |
| **Terpenes** | + | + |
| **Carbohydrate** | + | + |
| **Saponins** | - | + |

## 2.4 Thin Layer Chromatography

The TLC was used for the confirmation of compounds present in *Dendrophthoe falcata*. The procedure aimed to identify various compounds present in the *D. Falcata* leaf extract. The extract was mixed with a solvent (methanol and water) to dissolve the compounds. This mixture was then spotted onto a TLC plate coated with silica gel. The plate was placed in a chamber with a solvent mixture

(hexane and ethyl acetate). The result showed 3-4 distinct spots on the plate, indicating the presence of 3-4 different phytoconstituents in each of the subjected leaf sample. The interpretation of the TLC Analysis is depicted in Table 2.

**Table 2 - Interpretation For the TLC Analysis**

| ***Dendrophthoe falcata*** | **$R_f$ Value @ UV 254nm** | **Visible** | **$R_f$ Value @ UV 366nm** |
|---|---|---|---|
| **A** | **0.73** | **0.73** | **0.73** |
| **B** | **0.70** | **0.70** | **0.70** |
| **C** | **0.46** | **0.46** | **0.46** |
| **D** | **0.23** | **0.23** | **-** |

## 2.5 High-Performance Liquid Chromatography

C18 Column is used with a flow rate of 1.0 ml/min. Mobile Phase- A mixture of Acetonitrile and Water (85:15 ratio). λmax - 202 nm (Fig. 1). Based on the data, we can conclude that the sample contains multiple compounds with different retention times, indicating different chemical properties. The compounds with higher peak areas or heights are indicative of compounds present in higher concentrations.

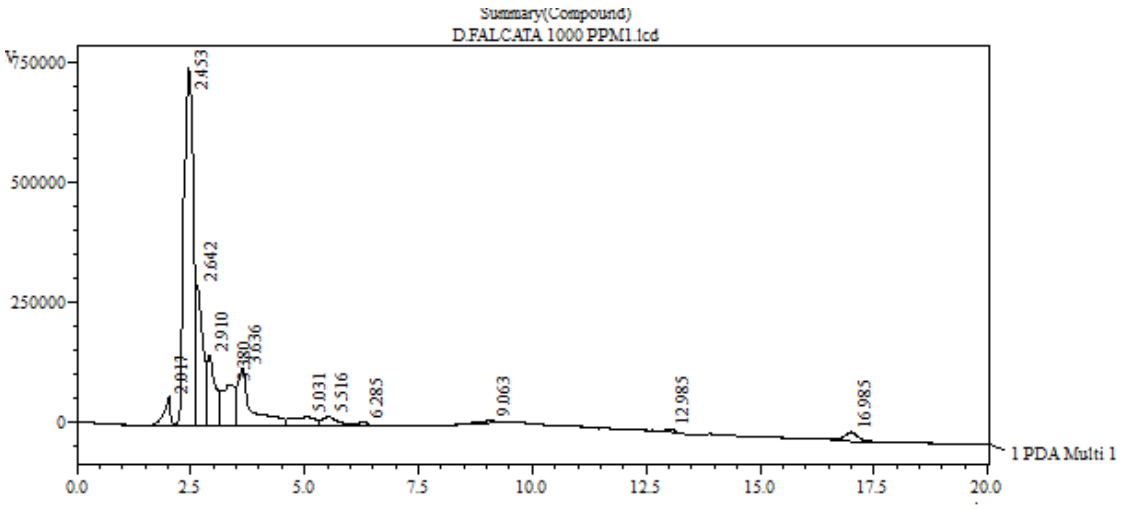


***Figure 1*** *- HPLC chromatogram of D. falcata 1000 PPM*

## 2.6 UV visible spectroscopy

The absorbance maximum of the *Dendrophthoe falcata* Methanol Extract was recorded at UV-Vis region (200-800 nm). It produced a clear peak at 205 nm for Stigmasterol (Fig. 2). The presence of a clear peak at 205 nm suggests the presence of a compound, Stigmasterol, which absorbs light strongly at this wavelength. Stigmasterol is a phytosterol, a type of steroid alcohol found in plants. It's known for its absorption in the UV region.

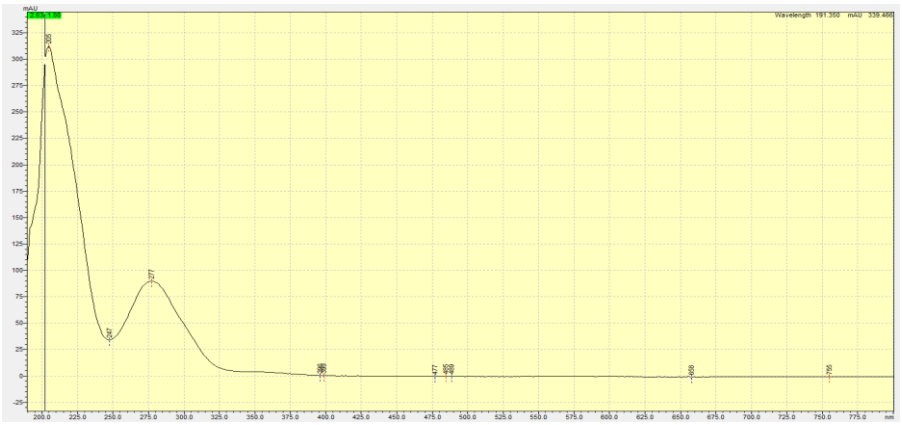

***Figure 2 -*** *UV visible Spectroscopy λmax value of Dendrophthoe falcata methanol extract*

## 2.7 Preparation of methanolic extract

Shade dried leaves of *Dendrophthoe falcata* were powdered and extracted using methanol by the Soxhlet extraction procedure. The Soxhlet Extraction prolonged for 6 hours, the received plant material weight was 250 gms. The sample taken for extraction weighed 150gms and Methanol taken for extraction was 1.2L. the Crude Extraction Weight after first extraction valued to be 1.5159g and that after second extraction valued 0.6597g, accounting for the Total Crude Weight to be 2.1756g. The collected extract was then concentrated in a rotary vacuum evaporator.

## 2.8 Acute toxicity study

An acute toxicity study of the methanolic extract of *Dendrophthoe falcata* leaves on adult zebrafishes (length 2.6 - 2.8cm, weight 0.2 - 0.3 g). was conducted with Lethal Concentration 50 (LC50). The study abided static water renewal experiment following OECD-203 criteria for chemical testing. Seven randomly chosen zebrafish of both sexes were subjected to varying doses of *Dendrophthoe falcata* leaves extract in a rectangular glass tank of 5 litres capacity. Five separate concentrations of *Dendrophthoe falcata* leaves (20, 40, 60, 80, 100 mg/L), each along with a control was used for this test. The test concentration of *Dendrophthoe falcata* leaves was chosen based on results from a range finding experiment. Fishes were classified as dead when operculum movement stopped and they couldn't respond to a glass rod. Deaths were regularly checked. The dead fish were right away taken out of the tank. Following 24 hours, the fish were transplanted to new tanks with different concentrations of *Dendrophthoe falcata* leaf extracts. The fishes were not fed before or throughout the trial period. Throughout the trial, the behaviour of the test fish was continuously monitored. OECD guidelines were followed to conduct acute toxicity tests.

### 1. Experimental design

A total of 50 adult zebrafishes with equal number of male and females were used for the drug exposure. The fishes were distributed equally into groups of five, with ten fishes in each group (Table 3). Group I was the unexposed Control Group with 10 zebrafishes that did not receive any drug exposure. The remaining forty zebrafishes belonging to the Group II, Group III, Group IV and Group V, were treated with Paracetamol (Sigma Aldrich A7085) 5mM (755.8 mg/L) for a period of 7 days in order to induce hepatotoxicity[15]. Group II were the Paracetamol Control Group which did not receive any therapeutic drug post intoxication. Group III zebrafishes received the test drug orally at the lower concentration (Table 1). Group IV received the test drug at a higher concentration along with paracetamol 5mM for the same period of 7 days. Group V received along with the paracetamol 5mM, the standard drug Silymarin (Sigma Aldrich S0292) with a dosage of 20µg/L. Zebrafishes were receiving oral dosing for a period of 168 hours (7 days) to develop an established result in their organs. The fish were put to death with an 80 ppm exposure to clove oil in the tank waters following a week of exposure[16].

Table 3 – Experimental Design

| Group | Treatment | n |
|---|---|---|
| I | Control group (Unexposed) | 10 |
| II | Paracetamol 5 mM (755.8 mg/L) | 10 |
| III | Paracetamol 5 mM + Extract (40 mg/l) | 10 |
| IV | Paracetamol 5 mM + Extract (60 mg/l) | 10 |
| V | Paracetamol 5 mM + Silymarin (20µg/L) | 10 |

## 2. Histopathology

Post Euthanasia, the zebrafish liver tissue was dissected and preserved with a 10% formalin solution at 4°C for duration of 24 hours. After being hyalinized with xylene and dehydrated with gradient ethanol, the connected liver tissues were embedded in paraffin wax at 56°C. Next, the 4µm-thick wax blocks were cut. Next, using a staining kit, the sections were put on glass slides and stained with hematoxylin and eosin (H&E). Histologic lesions were inspected using a digital camera-connected optical microscope.The liver tissues that were connected were hyalinized with xylene, dried with gradient ethanol, and then embedded in paraffin wax at 56°C. After that, the wax blocks were sliced at a thickness of 4µm. Following that, the sections were put on glass slides and stained using a staining kit that included hematoxylin and eosin (H&E). Histologic lesions were inspected using a digital camera-connected optical microscope.

## 3. Results and discussion

The dosage of paracetamol used for inducing hepatotoxicity being mild, did not result in deaths of any of the experimental fishes during the 7 days duration. Although the dose used did end up highlighting the early to mid stage hepatic damages caused to the zebrafishes. The parameters that were looked for in the histopathological analysis were condition of hepatocytes, portal triad structures, central vein and sinusoids. Both male and female zebrafishes were separately analysed for all the groups. The histopathology reports of Group I demonstrated normal hepatocytes, normal portal triad structures, central vein appeared normal, and sinusoids also appeared normal for both sexes (fig.3A, fig.4A). The Group II (Paracetamol Control) histopathology data portrayed considerable damage with difference in both sexes, the male zebrafishes showed mild degeneration in the periportal hepatocytes,

dilated sinusoids, Congested central vein with normal portal structures (fig.3B). Whereas among the female zebrafishes, besides the hepatic damages seen in the male fishes, periportal inflammation was also spotted (fig.4B). Both the female and male zebrafishes of Group III receiving the test drug (methanolic extract of *Dendrophthoe falcata* leaves) with a milder concentration of 40 mg/l demonstrated normal hepatocytes and portal triad structures, the central vein and sinusoids also appeared normal (fig.3C, fig.4C). This is suggestive that the 40 mg/l dosage of the test drug is highly effective in treating the hepatic damage caused by the exposure of 5mM paracetamol among adult zebrafishes of both sexes. Group IV received a stronger concentration of the test drug with 60 mg/l concentration but still the histopathological results of both Group III and Group IV were found to be similar when the accounting parameters of hepatocytes, portal triad structures, central vein and sinusoids are correlated (fig.3D, fig.4D). Group V Zebrafishes, which were receiving the conventional treatment with Silymarin (20µg/L) also depicted a normal histopathological report (fig.3E, fig.4E).

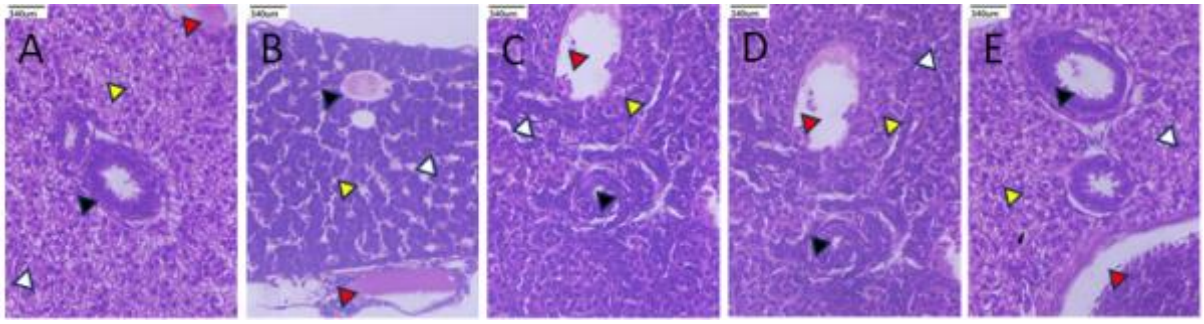


**Figure 3 -** Histopathological images of Adult Male Zebrafish Liver, A- Group I (Unexposed Control) - Normal, B- Group II (Paracetamol Control) – Shows liver damage, C- Group III (Extract 40mg/l) – Normal, D- Group IV (Extract 60mg/l) – Normal, E- Group V (silymarin 20µg/l) – Normal. Yellow Arrow- Hepatocytes, Red Arrow- Central Vein, Black Arrow- Portal triad structures, White Arrow- Sinusoids.

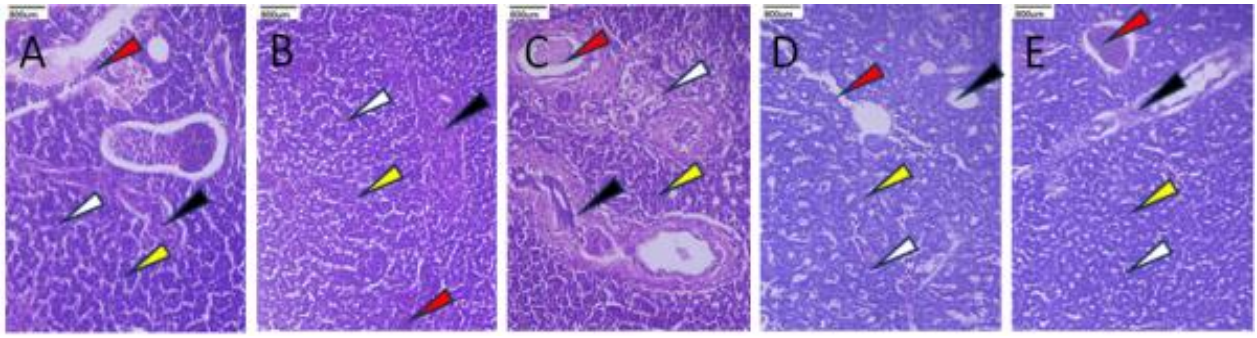


**Figure 4 -** Histopathological images of Adult Female Zebrafish Liver, A- Group I (Unexposed Control) - Normal, B- Group II (Paracetamol Control) – Shows liver damage, C- Group III (Extract 40mg/l) – Normal, D- Group IV (Extract 60mg/l) – Normal, E- Group V (silymarin 20µg/l) – Normal. Yellow Arrow- Hepatocytes, Red Arrow- Central Vein, Black Arrow- Portal triad structures, White Arrow- Sinusoids.

## 4. Conclusion

By the reference of all the experimental and analysed data we can draw a conclusion that the methanolic extract of *Dendrophthoe falcata* leaves in both the tested concentrations of 40mg/l and 60mg/l has proven hepatoprotective effect over paracetamol induced liver toxicity in adult zebrafish that is therapeutically equivalent to Silymarin in the treatment for the same. Hence, it is hereby

relevant to say that that methanolic extract of *Dendrophthoe falcata* leaves harbour potential therapeutic medicinal properties essential for treating hepatotoxicity. Furthermore, biochemical examination and molecular pathway analysis can be used to assess the complete potential of *Dendrophthoe falcata* leaves in hepatotoxicity models when utilized with various doses.

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**Conflict of Interests**: No conflicts of interest are reported by any of the writers.

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