Hepatoprotective and Antioxidant Activity of *Phoenix pusilla* Fruits Extract Against Paracetamol Induced Hepatotoxicity in Freshwater Fish *Cirrhinus cirrhosus*

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**Abstract:** Liver damage has recently emerged as one of the biggest issues in aquaculture; many farms are experiencing the "liver syndrome," which manifests as liver enlargement (up to two to three times its original size) and colour changes. One of the main causes of the disorders may be xenobiotic challenge brought on by drug addiction and environmental contamination. There are very few effective medications that can protect the liver from harm and/or aid in the regeneration of hepatic cells, despite the enormous breakthroughs in contemporary medicine. In the current investigation, fish *Cirrhinus cirrhosus* with paracetamol-induced hepatotoxicity were supplemented with *Phoenix pusilla* fruit extract. Its hepatoprotective efficacy was proven by the restoration of the liver indicators ALT, AST, protein, and histological changes. *Phoenix pusilla* fruit contains a large number of secondary metabolites, such as flavonoids, which may act in a number of different ways to shield the hepatocytes from harmful chemicals. Thus, the current study concluded that *Phoenix pusilla* fruit may effectively prevent liver damage from paracetamol by protecting it from oxidative damage and tissue-damaging enzyme activities.

**Keywords:** *Phoenix pusilla* fruit extract, Hepatoprotective activity, Liver markers, Paracetamol, *Cirrhinus cirrhosus*

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**Introduction**

The biggest gland, the liver, is a crucial organ. It serves as the body's "engine room" for metabolism. The liver is responsible for almost all drug metabolism and detoxification, and as a result, it is frequently exposed to diseases that cause a variety of clinical symptoms. A wide range of liver illnesses, including hepatitis, jaundice, cirrhosis, and liver cancer, can be caused by a number of chemicals, foods, medicines, and infections (parasitic, bacterial, viral, or fungal). Modern medications are mostly used to treat plant-based preparations for liver illnesses since
they have the most to offer in terms of improving the treatment of hepatic disease (Qureshi et al., 2007). For many years, natural products have been essential for health care. Algae are frequently varied sources of natural goods, but they are also a source of chemical substances that are used as standalone medications or as essential components in formulations incorporating synthetic pharmaceuticals. A key component of the investigation’s success is the choice of marine species (Watanuki et al., 2006). Although a focused collection based on chemotaxonomic linkages and marine knowledge collected from algae is more likely to provide pharmacologically active chemicals, random selection can provide some indications. There is still a growing need for algae therapies despite the enormous advancements in contemporary medicine. For effective and potent algal therapies to be certified for the treatment of illnesses, they must undergo standard scientific review. The important health issue of medication-induced liver toxicity confronts not only medical practitioners but also the pharmaceutical business and drug regulatory organisations. Inhibiting the production of free radicals can be used as a simple model to assess the effectiveness of hepatoprotective drugs (Saleem, 2008). Traditional herbalists and native healers across the world have employed botanicals to prevent and treat liver illness (Luper, 1998). The objective of the current study was to assess *Phoenix pusilla* fruit extract’s hepatoprotective and antioxidant efficacy against paracetamol-induced hepatotoxicity in freshwater fish *Cirrhinus cirrhosus*

**Materials and Methods**

*Collection and acclimation of experimental fishes:*

*Cirrhinus cirrhosus* (Mrigal carp) (3.50±0.43 g) were procured using a cast net from a fish farm in Thittai, Thanjavur District, Tamil Nadu, India. They were kept in a glass aquarium tank in the laboratory and acclimated in aerated tap water with continuous aeration for two weeks before the experiment. Fish were fed a known quantity of fish meal throughout acclimatization.

**Experimental design:**

Fish were separated from the stock and acclimated for 3 days to the lab settings (temperature 28°C, pH 7.5-7.8, and an essentially normal photoperiod 12:12-h L/D). The fish were of uniform size (5.35 cm in length and 3.50 g in weight). The fish were divided into three groups of ten each-- one control group and two experimental groups. They were kept into a trough filled with dechlorinated tap water. The appropriate substance was given orally to all of the experimental fish. Fish in Group I were given 0.09% saline as a control, Group II received a single dosage of paracetamol (500 mg/kg body weight), and Group III received paracetamol (500 mg/kg body weight) with an aqueous extract of *Phoenix pusilla* fruits (250 mg/kg bw). *Phoenix pusilla* fruit powder weighing 5 g was added into a conical flask (250 ml) having 200 ml aqueous solvent. *Phoenix pusilla* fruits were shaken vigorously for 30 min. The extracts were filtered using Whatman filter paper No. 1 after 24 h, and the filtrate was kept for further use. Extract from *Phoenix pusilla* fruits were given to the fish after 30 min of paracetamol. Before being administered, the paracetamol was first dissolved in water at 70°C and then cooled to 37°C. The fish were sacrificed after 24 h. For biochemical analysis, liver tissue samples were processed.

Blood from the caudal vein, with and without a heparinized 2 ml disposable syringe connected with a 21-gauge hypodermic needle was taken at the end of the experiment. The blood was cooled for 30 min and then kept at room temperature to allow the blood to coagulate. Blood was centrifuged for 10 min at 3000 rpm, the serum (supernatant) was separated and frozen until needed for antioxidant and lever function analysis.

**Tissue homogenate:**

After sacrificing the fish, liver were removed and cleaned with ice-cold physiological saline. A Teflon homogenizer was used to weigh and homogenise the needed quantity. 0.1 M Tris HCl buffer (pH 7.4)
was used to create tissue homogenate, which was then utilized to estimate biochemical parameters.

**Biochemical Estimation:**

The Beuge and Aust (1978) thiobarbituric acid test technique was used to measure malondialdehyde. The Moron et al. (1979) technique was used to determine reduced glutathione. The Rotruck et al. (1973) technique was used to measure the glutathione peroxidase. The method of Kakkar et al. (1984) was used to determine the superoxide dismutase activity. Rangarajan et al. (2021) technique was used to measure the activity of catalase. The Reitman and Frankel (1957) technique was used to estimate the serum GOT. The Reitman and Frankel (1957) technique was used to estimate the serum GPT. Using the Lowry et al. (1951) technique protein was calculated. Ochei and Kolhatkar (2000) method was used for histological analyses of the liver.

**Phoenix pusilla fruit phytochemical analysis:**

**Collection of plant and preparation of extract:**

Fully grown Phoenix pusilla fruits were harvested in June 2022 from Kathattiipattii (Palaiyappatti North), Sengipatti, Thanjavur District, Tamil Nadu, India. Phoenix pusilla fruit powder weighing 5 g was added into a conical flask (250 ml) containing 200 ml aqueous solvent. Phoenix pusilla fruits were shaken vigorously for 30 min. The extracts were filtered using Whatman filter paper No. 1 after 24 h, and the filtrate was used for further investigation.

**Phytochemical analysis:**

Standard protocols were used to conduct chemical analyses on the extract to identify the ingredients.

**Statistical analysis:**

The SPSS Software version 20 was used to analyze the results. Three replicates were conducted in each group, and data were represented as Mean ± SD. One Way Analysis of Variance (ANOVA) and Duncan’s multiple range test were used to identify significant differences between mean values (DMRT). It was deemed significant at P< 0.05.

**Results**

The levels of MDA, GSH, SOD, CAT, and GPx in the liver tissue and plasma of the control and experimental groups of Cirrhinus cirrhosus are shown in Figures 1 and 2. Compared to group I, group II had a higher MDA content. The difference in the MDA content between groups III and I was not statistically significant. Activity levels of GSH, SOD, catalase, and glutathione peroxidase were lower in group II than in group I. The amount of GSH, SOD, catalase, and glutathione peroxidase in group III were not statistically different from group I.

![Fig. 1: Plasma antioxidant and stress marker in control and experimental fish of Cirrhinus cirrhosus (n= 10).](image)

Different letters (superscript) are statistically significant (P<0.05) from each other group and same letters are statistically non-significant (P>0.05) are compared by one-way ANOVA, followed by post-hoc Tukey HSD test, significant level alpha 0.05.

The plasma concentrations of AST, ALT, and protein in the control and experimental groups of Cirrhinus cirrhosus fish are shown in Figure 3. Comparing group II to group I, there was an increase in SGOT and SGPT activity. Supplementing fish with Phoenix pusilla fruit
Fig. 2: Liver antioxidant and stress marker in control and experimental fish of *Cirrhinus cirrhosus* (n=10). Different letters (superscript) are statistically significant (P<0.05) from each other group and same letters are statistically non-significant (P>0.05) are compared by one-way ANOVA, followed by post-hoc Tukey HSD test, significant level alpha 0.05.

extract led to non-significant increases in protein, SGOT, and SGPT in group III compared to group I.

Histopathological observations of liver of Group II fish revealed that paracetamol increased the gap and made the liver's derangement irregular. Hepatocellular vacuolization and degeneration were seen in the liver cells. The control (group I) showed the liver's normal structure. Figure 4 depicts the liver architecture of Group III supplemented with Phoenix pusilla fruit extract showing minor vacuolization.

**Phytochemical analysis:**

The *Phoenix pusilla* fruit extract used in the current investigation was found to have components that have therapeutic value. Table 1 summarises the phytochemical characteristics of the fruit of the *Phoenix pusilla* plant. In contrast to the absence of alkaloids in the aqueous extract, tannin, saponins, flavonoids, steroids, terpenoids, triterpenoids, polyphenols, glycosides, and coumarins were present.

Fig. 3: The levels of liver function in AST, ALT and protein in control and experimental fish. Different letters (superscript) are statistically significant (P<0.05) from each other group and same letters are statistically non-significant (P>0.05) are compared by one-way ANOVA, followed by post-hoc Tukey HSD test, significant level alpha 0.05.

**Discussion**

The second most common reason for liver transplantation and a major source of morbidity and death is paracetamol-induced hepatic failure (Lee, 2004). A common analgesic and antipyretic medication is paracetamol. It produce hepatotoxicity in humans and laboratory animals when consumed in excess, which raises liver enzyme levels (Kumar *et al.*, 2004a). Cellular enzyme leakage into plasma is an indicator of liver damage or injury (Kumar *et al.*, 2004b). Additionally, the presence or absence of certain enzymes in the circulation can be used to determine the degree and type of liver damage or injury (Kumar *et al.*, 2005). In general, measures of the marker enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are frequently employed to evaluate the hepatotoxicity caused by paracetamol (Yen *et al.*, 2007). We discovered that 24 h after paracetamol
exposure, the activity of the tissue-damaging enzymes AST and ALT significantly increased in *Cirrhinus cirrhosus*. Enzymes involved in the metabolism of amino acids include AST and ALT. It has been demonstrated that an increase in ALT and AST values indicates liver injury (Bhattacharya *et al.*, 2005). Determining the levels of enzymes like AST and ALT is frequently utilized in the evaluation of liver damage caused by paracetamol. The enzyme is released into circulation via necrosis or membrane damage and may thus be detected in the serum. All cells contain the enzyme AST in their mitochondria and cytoplasm. High AST values also signal myocardial infarction and muscular injury in addition to liver damage from viral hepatitis. AST is released similarly and catalyses the conversion of glutamate and alanine to pyruvate. A rise in the cytoplasmic hepatocellular enzyme ALT in the blood is a strong indicator of liver disease, such as cirrhosis, hepatitis, or hepatic tumours. As a result, ALT is an important measure for identifying liver damage since it is more specific to the liver. Increased enzyme levels in the liver are a sign of cellular leakage and a loss of the functional integrity of the cell membrane. Treatment with *Phoenix pusilla* fruit considerably decreased the increased AST and ALT activities.

The current findings demonstrated that paracetamol lowers the amounts of proteins in the liver. Total protein concentrations in human tissues and the rate of protein production in liver tissue are closely correlated. The decreased protein production in the liver is reflected by the low amount of total protein in the liver, which is made up of albumin and globulin. *Phoenix pusilla* fruit supplementation resulted in a significant rise in plasma proteins. In this study the enzyme levels of MDA, GSH, SOD, CAT, and GPx were significantly elevated in the liver tissue and plasma of the control and experimental group of *Cirrhinus cirrhosus* fish after receiving a single dosage of paracetamol (500 mg/kg bw). Compared to group I, group II had a higher MDA content. The difference in the MDA content between groups III and I was not statistically significant. Activity levels of GSH, SOD, catalase, and glutathione peroxidase were lower in group II than in group I. The amount of GSH, SOD, catalase, and glutathione peroxidase found in group III was not statistically different from group I. *Phoenix pusilla* fruit’s elevated enzyme levels in liver caused by paracetamol may be attributable to the fruit’s antioxidant and membrane-stabilizing properties, which stop intracellular enzyme leakage.

The levels of all the indicated enzymes were restored to normal or close to normal. The small scope of histological alterations further corroborated this. In physiopathological circumstances involving lipid peroxidation events, free radicals play significant role. It is known that paracetamol undergoes conversion via the cytochrome P450
route to the very poisonous metabolite acetyl-p-
benzoquinone imine (Sounder et al., 2018) which
is typically conjugated with glutathione and
eliminated in the urine. Paracetamol overdose
depletes glutathione reserves, which builds up
NAPQI mitochondrial malfunction and causes
acute liver necrosis (Parmar and Kandakar, 1995).
It is well known that a number of P450 enzymes
are crucial for the bioactivation of paracetamol to
NAPQI. The major enzymes for paracetamol
bioactivation in liver microsomes may be P450
2E1 (Raucy et al., 1989). Studies showed that
compounds that affect P450 activity can control
the hepatotoxicity caused by paracetamol
(Mitchell et al., 1973). According to Wendel et al.
(1982), the metabolism of paracetamol causes
lipid peroxidation, which results in liver damage.
SOD is the first enzyme to deal with oxyradicals
and catalyses the conversion of superoxide
radicals to \( \text{H}_2\text{O}_2 \) and \( \text{O}_2 \) (Kappus, 1985). In
response to oxidative stress, SOD activity had a
counterproductive effect. In this study,
paracetamol administration dramatically
increased SOD activity, indicating an increase in
superoxide radical anion. When treated with 500
mg/kg bw of paracetamol, SOD reacted to the
oxidative stress, indicating that its activity was
extremely sensitive. As a result, enhanced ROS
generation may be responsible for the SOD and
CAT induction shown in our study. Increased
antioxidant status makes an effort to reduce ROS’s
negative effects. All animal tissues include
peroxidases, which are enzyme antioxidants that
break down hydrogen peroxide and shield the
tissue from extremely dangerous hydroxyl
radicals. As a result of the buildup of superoxide
radicals and hydrogen peroxide, the decreased
activity of these enzymes may have a range of
harmful effects (Kumar et al., 2005). Damage
results from oxidative stress, which happens when
the delicate balance between oxidants and
antioxidants is upset owing to the depletion of
antioxidants, an excessive buildup of ROS, or both
(Scandalios, 2005). The most sensitive enzymatic
marker for liver injury is an increase in SOD
enzyme activity, which is a sensitive indicator of
hepatocellular damage (Curtis and Mortiz, 1972).
One of the most crucial enzymes in the enzymatic
antioxidant defence system is SOD. It lessens the
cellular's harmful effects by scavenging the
superoxide anion to produce hydrogen peroxide.
Phoenix pusilla fruit significantly lowers hepatic
SOD activity which lessens reactive free radical-
induced liver oxidative damage. One of the most

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Phytochemicals</th>
<th>Aqueous extract</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Tannin</td>
<td>++</td>
</tr>
<tr>
<td>2</td>
<td>Saponin</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Flavonoids</td>
<td>++</td>
</tr>
<tr>
<td>4</td>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Terpenoids</td>
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</tr>
<tr>
<td>6</td>
<td>Triterpenoids</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Alkaloids</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Antroquinone</td>
<td>++</td>
</tr>
<tr>
<td>9</td>
<td>Polyphenol</td>
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</tr>
<tr>
<td>10</td>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>Coumarins</td>
<td>++</td>
</tr>
</tbody>
</table>

(+) Present, (+++) High concentrations and (--) Absent
prevalent tripeptide, nonenzymatic biological antioxidants in the liver is glutathione. It preserves membrane protein thiols and eliminates free radical species like hydrogen peroxide and superoxide radicals. It serves as a substrate for GPx (Haracska et al., 2001). By joining with the reactive metabolites of paracetamol, GSH plays a crucial part in the detoxification of paracetamol and protects hepatocytes. As a result, it stops them from forming a covalent bond with liver proteins. Intracellular decline of GSH exposes the cell to oxidative stress’ harmful consequences (Lauterburg and Velez, 1988). In fish given paracetamol, there is a correlation between the GSH level’s decline and an increase in lipid peroxidation. Treatment with Phoenix pusilla fruit considerably raised the high GSH levels. Studies on the histopathology of liver tissue treated with paracetamol revealed a clear disorganization of muscle bundles, becoming asymmetrical with a wider gap. Hepatocellular vacuolization and degeneration were seen in the liver cells. In the fish treated with Phoenix pusilla fruit, the aforementioned abnormalities were successfully reduced.

Modern medicine still faces difficulties in treating liver conditions. Only corticosteroids and immunosuppressive medications are available to treat liver disorders, and both of these have a number of dangerous side effects. Thus there is need for complementary and alternative medicine, particularly herbal and plant-based remedies. Since they are readily available in nature, cheap, and have few to no side effects, medicinal plants are essential for the therapy of liver disorders in the poor countries (Sheetal and Singh, 2008).

Several medicinal plants including the stem of Leptadenia reticulata (Amit et al., 2011), the roots of Suaeda fructocosa (Rehman et al., 2013), the leaves of Cleome viscosa (Gupta and Dixit, 2009), Zingiber officinale, and the berries of Piprum nigrum (Rehman et al., 2013; Jamil et al., 2013) supported our study. Our study analyzed the phytochemical components of Phoenix pusilla fruit extract which showed presence of tannin, saponins, flavonoids, steroids, terpenoids, triterpenoids, polyphenols, glycosides, antroquinones, and coumarins.

**Conclusion**

Overall, the lipid peroxidation inhibition and antioxidant enzyme level elevation together with the free radical scavenging activity of Phoenix pusilla fruit appear to be connected to the protective impact on paracetamol-induced hepatotoxicity in fishes. Phoenix pusilla fruit has a large number of secondary metabolites, such as flavonoids, which may work in a number of different ways to shield the hepatocytes from harmful chemicals. The present study concluded that Phoenix pusilla fruit can effectively prevent liver damage from acetaminophen by protecting the organ from oxidative stress and tissue-damaging enzyme activity.

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