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Anti-inflammatory effect of Ficus benghalensis extract against acrylamide induced inflammation in Zebrafish larvae

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Abstract: Introduction
Ficus benghalensis, commonly known as banyan, is a tree that has been shown to have anti-inflammatory properties. The tree contains several bioactive compounds, including polyphenols, flavonoids, and carotenoids, which are believed to contribute to its anti-inflammatory effects. In addition, some studies have suggested that Ficus benghalensis may have a protective effect against inflammatory bowel disease (IBD), a chronic inflammatory disorder of the gastrointestinal tract. In animal models of IBD, treatment with banyan extract has been shown to reduce inflammation and improve the integrity of the intestinal barrier. In this study the antiinflammatory effect of FB extract was investigated in the acrylamide exposed zebrafish larvae. The FB extract reduced the neurotoxicity response in the zebrafish embryos by decreasing the oxidative stress condition. The antioxidant defense levels are upregulated and this condition showed that FB extract normalized condition. The AChE levels are maintained in the embryos exposure after the treatment. These results suggest that FB extract can be used as the protective agent.

Keywords: Ficus benghalensis, inflammation, antioxidant, zebrafish larvae.

1. Introduction

Intestinal inflammation is characterized by the infiltration of immune cells into the intestinal tissue, leading to tissue damage and dysfunction [1,2]. In animal models of acrylamide-induced intestinal inflammation, exposure to acrylamide has been shown to cause an increase in the production of pro-inflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), as well as an increase in the number of immune cells infiltrating the intestinal tissue [3,4]. The mechanism by which acrylamide induces intestinal inflammation is not fully understood, but it is thought to involve the activation of the nuclear factor-kappa B (NF-κB) signaling pathway. NF-κB is a transcription factor that plays a key role in the regulation of inflammation and immune responses. Exposure to acrylamide has been shown to activate NF-κB signaling in intestinal cells, leading to the production of pro-inflammatory cytokines and the recruitment of immune cells to the site of inflammation [5–7]. To study acrylamide-induced intestinal inflammation in zebrafish, researchers have used a range of techniques, including histological analysis, gene expression analysis, and imaging of immune cell infiltration using fluorescent markers [8–10]. These studies have provided important insights into the mechanisms underlying acrylamide-induced intestinal inflammation, and have helped to identify potential targets for intervention and treatment.

Several studies have investigated the anti-inflammatory activity of *Ficus benghalensis* in various models, including animal models and cell culture studies [11–13]. For example, one study found that an extract of banyan flower had significant anti-inflammatory activity in mice with induced inflammation, as evidenced by a reduction in the levels of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 [14–16]. Another study investigated the effects of banyan extract on human white blood cells (leukocytes) in vitro, and found that the extract was able to suppress the production of pro-inflammatory cytokines, including TNF-α and IL-6 [17–19]. This suggests that banyan extract may have a direct anti-inflammatory effect on immune cells involved in inflammation. In addition, some studies have suggested that *Ficus benghalensis* may have a protective effect against inflammatory bowel disease (IBD), a chronic inflammatory disorder of the gastrointestinal tract. In animal models of IBD, treatment with banyan extract has been shown to reduce inflammation and improve the

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integrity of the intestinal barrier. In this study, the protective effect of FB extract was investigated in the intestinal inflammation induced zebrafish larvae.

2. Materials And Methods

II a. Origin and maintenance of zebrafish

Adult zebrafish (Wild type – AB strain, 4 months old) were purchased from a local aquarist (NSK aquarium, Kolathur, Tamil Nadu, India). The male and female fishes were separated, maintained in our facility under the following condition in a 10 L glass tank: 28.5˚C, with a 14/10 h light/dark cycle [20–22]. The fish were fed three times a day, with live brine shrimp (*Artemia salina*) [20–22]. The fishes were acclimatized for 1 month, later the fishes were utilized for breeding, and larvae were collected and used for the following experiments [23–25]. The collected larvae are further analyzed under a microscope, unfertilized larvae are discarded, whereas the fertilized larvae are taken in a six-well plate and incubated in E3 medium [4,16,26].

II b.Protective effect analysis in zebrafish larvae

For the protective effect assessment studies, 4 hpf larvae were used, the exposure was carried in a 6 well plate containing untreated larvae as the control, and with different FB extract concentration (2.5 mg/mL and 5mg/mL) [27–29]. Around 15 larvae/well were used with 3 mL of E3 medium. The exposure was non-static and renewed every 24 h with the fresh treatment solution throughout the exposure period (4 hpf to 96 hpf). All the experiments were carried out in triplicates. Parameters such as survival and heart rate were observed during this period, and calculations were presented at the end of 96 hpf [6,30,31].

II c. GPx assay in larvae

To perform the assay, zebrafish larvae are first homogenized in a lysis buffer to release the GPx enzyme from the cells [32–37]. The homogenate is then centrifuged to remove cellular debris, and the resulting supernatant is used as the enzyme source for the assay [38–41]. The assay mixture, containing the substrate, GSH, and cofactor, is added to the supernatant, and the reaction is allowed to proceed for a specific amount of time. The reaction is then stopped, and the concentration of the colored or fluorescent product is measured using a spectrophotometer or fluorometer.

II d. NO production in larvae

One common method for measuring NO levels is the Griess assay, which involves the reaction of NO with sulfanilic acid and naphthylethylenediamine dihydrochloride to form a colored azo compound. To perform the assay, zebrafish larvae are homogenized in a buffer solution, and the supernatant is mixed with the Griess reagent. After incubation, the absorbance of the colored product is measured using a spectrophotometer, with the amount of NO present in the sample being proportional to the absorbance [7,42–44].

II e. Lipid peroxidation assay in larvae

To assay lipid peroxidation in zebrafish larvae, a common approach is to use a colorimetric or fluorometric assay kit that measures the levels of malondialdehyde (MDA), a byproduct of lipid peroxidation. One common method for measuring MDA levels is the thiobarbituric acid reactive substances (TBARS) assay, which involves the reaction of MDA with thiobarbituric acid to form a colored product that can be measured spectrophotometrically. To perform the assay, zebrafish larvae are first homogenized in a buffer solution to extract the lipid-containing membranes. The homogenate is then incubated with thiobarbituric acid at high temperature, which results in the formation of a colored complex that can be measured spectrophotometrically [35,37,41].

II f. Statistical analysis

The data were presented as the mean of triplicates with a standard deviation. GraphPad Prism software (Ver 5.03, CA, USA) was used for statistical analysis. One-way ANOVA was performed and Tukey's post-hoc test was used to find levels of significance between control and other groups.

3. Result And Discussion

III a. Protective effect of MP extract

The FB extract of different concentrations (2.5 mg/mL and 5mg/mL) was treated to the larvae exposed to ACR (1 mM) to analyze their protective effect. The FB extract with different concentration was incubated with the larvae for 24 hrs. The results showed that ACR exposed larvae were dead. The treated larvae with FB extract were observed with normal morphology when compared to the control (Fig. 1).

III b.Survival and Heart rate

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Zebrafish larvae were widely used in the toxicity experiment and based on their survival and heart rate the toxicity effect was calculated. In this experiment, the untreated larvae was used as control and the treated with different groups of FB extract concentration (2.5 mg/mL and 5 mg/mL). The FB extract treatment showed the changes in the survival rate of larvae when compared to control. The survival rate was significantly increased ($p < 0.05$) to be 53% and 73% for the concentration of 2.5 mg/mL and 5 mg/mL.

Meanwhile, the heart rate of all the groups showed significant changes compared to the control group (Fig. 2). These results suggest that FB extract showed the protective effect

III c. Antiinflammatory activity of MP extract

Glutathione peroxidase (GPx) is an important antioxidant enzyme that plays a critical role in protecting cells from oxidative stress. GPx catalyzes the reduction of hydrogen peroxide and organic hydroperoxides using glutathione as a substrate, thereby preventing the buildup of reactive oxygen species (ROS) that can damage cellular structures and molecules. Lipid peroxidation is a process that results in the oxidative degradation of lipids, which can lead to the formation of reactive oxygen species (ROS) and the destruction of cellular membranes. To assay lipid peroxidation in zebrafish larvae, a common approach is to use a colorimetric or fluorometric assay kit that measures the levels of malondialdehyde (MDA), a byproduct of lipid peroxidation.

In this study, significant ($p < 0.05$) increases in total GPx and NO production was observed in the ACR exposed zebrafish larvae treated with FB extract (2.5 mg/mL and 5 mg/mL) compared to the control (Fig. 3). Zebrafish larvae treated with high concentration (5 mg/mL) of FB extract, showed elevated GPx (14 U/mg protein) and low NO (31µmol/mg protein) when compared to other groups. The GPx (4 U/mg protein) and high NO (62 µmol/mg protein) activity was significantly inhibited in the ACR (1mM) treated group

Nitric oxide (NO) is a signaling molecule that plays important roles in various physiological processes, including development, immune response, and neurotransmission. To assay NO levels in zebrafish larvae, there are several approaches available, including chemical and fluorescent-based assays. In this study, significant ($p < 0.05$) increases in LPO level was observed in zebrafish larvae treated with FB extract (2.5 mg/mL and 5 mg/mL) compared to the control (Fig. 4). Zebrafish larvae treated with high concentration (5 mg/mL) of FB extract, showed decreased LPO level (42 µmol/mL) when compared to other groups (Fig. 4). The AChE level (85 µmol/mL) was significantly inhibited in the ACR treated group.

4. Conclusion

From this study, it indicates that FB extract has antiinflammatory activity. It helps to maintain the antioxidant enzyme level during the oxidative stressed condition. The GPx level are upregulated during FB extract treatment. Also the FB extract didn't show any toxic level in the tested concentration and downregulated LPO and NO level.

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Figure 1.

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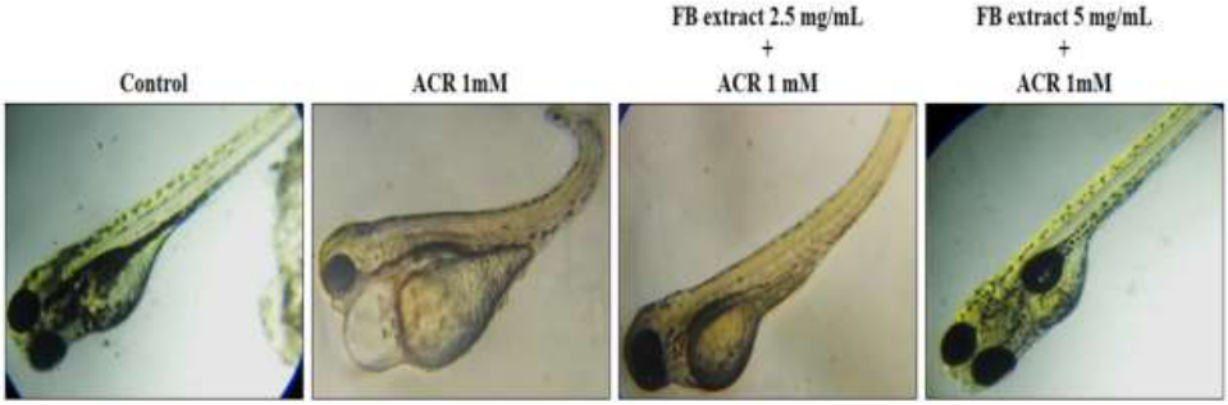

Fig. 1. Larvae were observed with normal morphology without any deformities when treated with FB extract. Control were untreated larvae.

Figure 2.

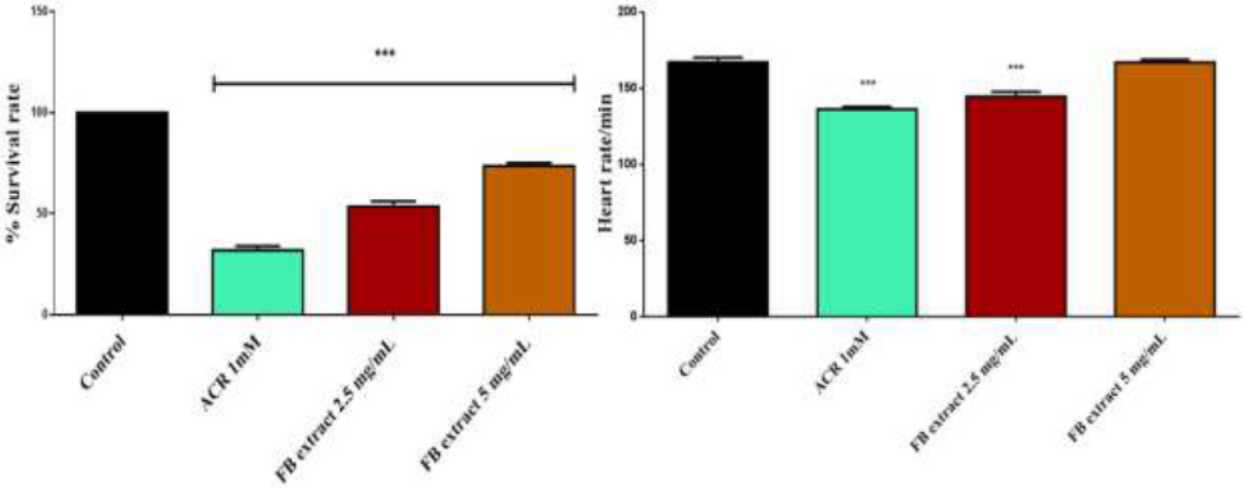

Fig. 2. The survival and Heart rate of zebrafish larvae was investigated after the exposed with different groups of FB extract for 24hrs. The ** ($p > 0.001$) indicates the significant difference between the control (untreated larvae) and treated group

Figure 3.

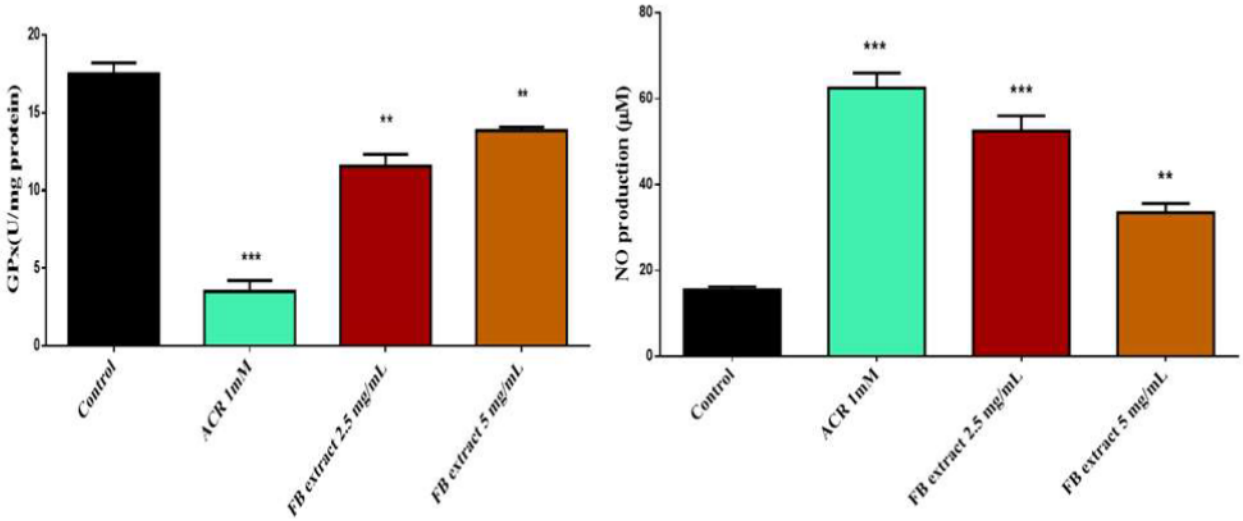

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Fig. 3. The GPx and NO activity in ACR induced neurotoxicity in zebrafish larvae treated with FB extract. Data expressed as mean ± standard deviation (n = 15/group). Values are statistically significant at *** p < 0.01 & ** *p < 0.001* compared to the control.

Figure 4.

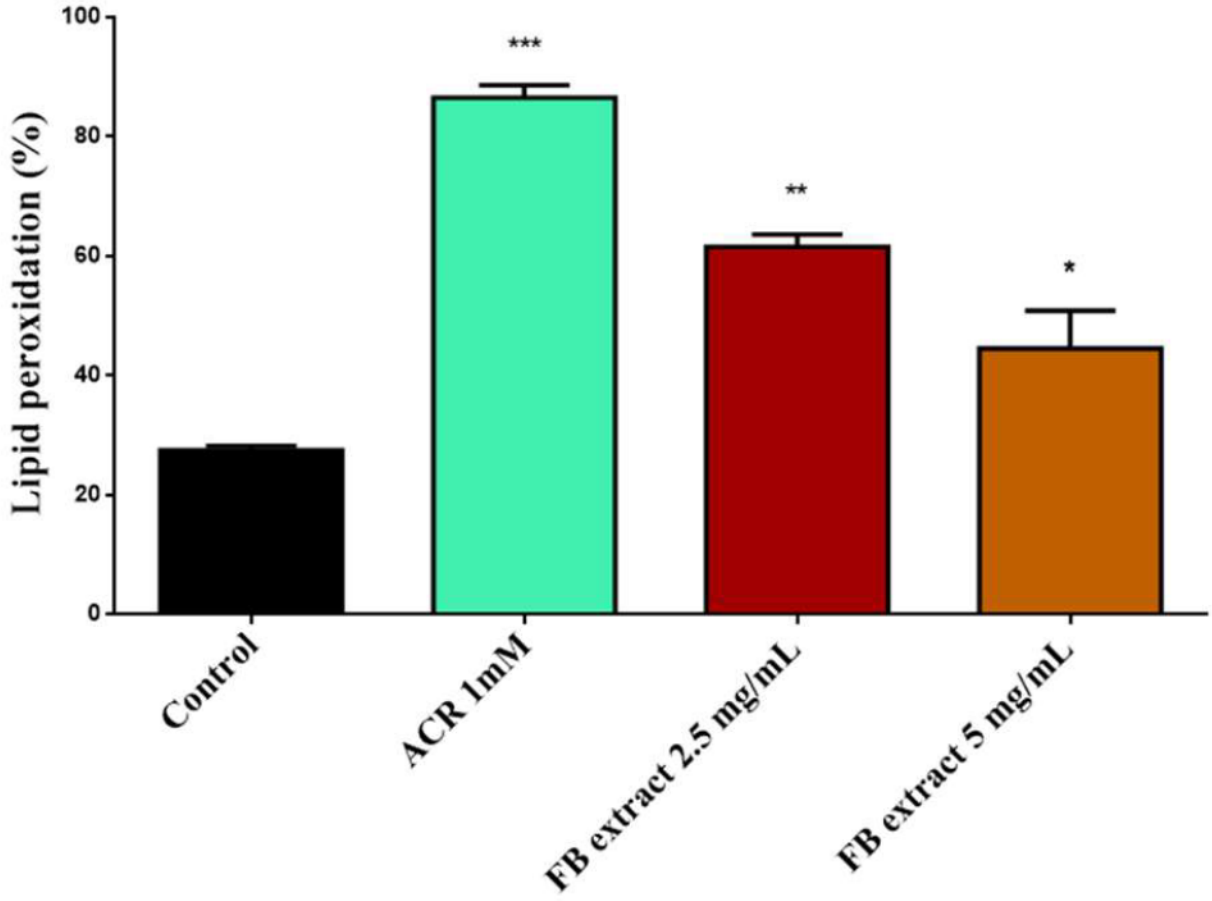

Fig. 4. The AChE level in ACR induced neurotoxicity in zebrafish larvae treated with FB extract. Data expressed as mean ± standard deviation (n = 15/group). Values are statistically significant at *** p < 0.01 & ** *p < 0.001* compared to the control.

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