INVESTIGATION OF ANTI-INFLAMMATORY EFFECT OF SELECTED PLANT EXTRACTS AGAINST *Escherichia coli* INDUCED SEPTIC SHOCK IN ZEBRAFISH MODEL

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Inflammation is initiated by the immune system in response to various adverse stimuli, such as infections, cellular damage, etc., protecting the host by recognizing and responding to these intrinsic and extrinsic factors by eliminating them and activating sophisticated and finely tuned mechanisms. Anti-inflammatory drugs are widely used to reduce inflammation though they have many harmful side effects. This study focused on evaluating the anti-inflammatory potential of Cinnamon *(Cinnamomum verum)*, Red sandalwood *(Pterocarpus santalinus)*, and Black pepper *(Piper nigrum)* extracts against *Escherichia coli*-induced inflammation in zebrafish larvae. Cold maceration with ethanol followed by concentration and freeze-drying were used to prepare the extracts. The highest concentration of each extract with no toxicity to zebrafish larvae which can be used as a drug in the drug screening study was determined. These concentrations were as follows; cinnamon- 20 µg/mL, pepper- 1000pg/mL, red sandalwood- 50 µg/mL, and stock solutions were prepared at these concentrations. Then Zebrafish larvae were exposed to a dilution series of stock solutions of herbal extracts as test material and 10^8 CFU/mL *E. coli* as an inflammatory agent with proper controls. Biomarker analysis included malformations, heartbeat, and macrophage migration assessment. *E. coli* infection significantly triggered inflammatory responses in zebrafish larvae, leading to septic shock-induced mortality. Malformations that were observed are necrotic yolk, bent spine, and swollen pericardial sac. Notably, the plant extracts, black pepper, and cinnamon significantly reduced the *E. coli* -induced inflammation. *E. coli* treatment increased the heart rate up to 263.00 ± 16.21 compared to the untreated control (161.80 ± 2.95). Posttreatment with black pepper 1000pg/mL and cinnamon 20 µg/mL reduced the *E. coli*-induced heart rate to 165.50±2.41 and 162.20±5.26, respectively. Neutral Red *E coli* treatment increased the NR accumulation up to 111.43 ± 1.66% compared to the untreated control (100 ± 4.14%). Posttreatment with black pepper and cinnamon reduced the *E. coli*-induced NR accumulation to 97.70±0.87 and 115.02±2.53, respectively. The highest concentration of cinnamon (20 µg/mL) and black pepper (1000 pg/mL) completely restored the normal phenotype. These findings highlight the potential of zebrafish as a rapid drug screening model, especially in evaluating plant-based anti-inflammatory interventions, and suggest the therapeutic promise of plant extracts in alleviating inflammation. Due to time constraints, we were unable to complete the experiment on Red Sandalwood. However, the data collected thus far is promising and warrants further investigation.

Keywords: *Anti-inflammatory drugs, Animal model, Drug screening model, Escherichia coli-induced inflammation, Macrophage migration*