

Investigation of anti-inflammatory effect of *Cinnamomum verum* and *Piper nigrum* extracts against *Escherichia coli* induced inflammation in zebrafish model

DMCB Dissanayake¹, IMN Molagoda², S Hettiarachi¹

Introduction and Objectives: Inflammation is a defense mechanism initiated by the immune system in response to various adverse stimuli, including infections and cellular damage. It protects the host by recognising and responding to intrinsic and extrinsic factors by activating sophisticated, finely tuned mechanisms. Anti-inflammatory drugs are widely used to reduce inflammation despite their many harmful side effects. This study evaluated the anti-inflammatory potential of cinnamon (*Cinnamomum verum*) (Sri Gemunu variety) and black pepper (*Piper nigrum*) (Panniyur 1 variety) extracts against *Escherichia coli*-induced inflammation in zebrafish larvae.

Methods: Cold maceration with ethanol followed by concentration and freeze-drying were used to prepare the extracts. Third day post-fertilisation (3dpf) zebrafish larvae (n=10) were exposed to different concentrations of herbal extracts and determined three nontoxic concentrations to the zebrafish model as cinnamon 20, 10, 5 µg/mL, and pepper 1000, 100, 10 pg/mL. The 3dpf zebrafish larvae were exposed to 10⁸ CFU/mL *E. coli* as the inflammatory agent. The post-treatment infected 4dpf larvae were treated with selected concentrations of plant extracts as test drug material. Biomarker analysis included malformations, heartbeat, and macrophage migration assessment. The neutral red (NR) staining experiment relative densities were quantified by Image J Software. The data were analysed using one-way ANOVA with Bonferroni correction.

Results: *E. coli* infection significantly triggered inflammatory responses in zebrafish larvae, leading to inflammation-induced mortality. Malformations observed were necrotic yolk, bent spine, and swollen pericardial sac. The following table shows the data on heart rate and NR accumulation and compares the effect of the post-treatment with the control. The plant extracts reduced both parameters to a level not significantly different from the control.

Parameter	Control	Treatment		
		<i>E. coli</i> alone	Black pepper	Cinnamon
Heart rate (beats/ min.)	161.80 ± 2.95 ^a	263.00 ± 16.21 ^b	165.50 ± 2.41 ^a	162.20 ± 5.26 ^a
NR Accumulation	100 ± 4.14% ^a	111.43 ± 1.66% ^b	97.70 ± 0.87% ^a	107.87 ± 3.61% ^a

The differences of values denoted by different letters in a row are statistically significant (p = < 0.001).

Conclusions: These findings reveal the anti-inflammatory potential of cinnamon and black pepper extracts. This research opens promising avenues for developing effective, natural plant-based anti-inflammatory treatment. The importance of zebrafish model in evaluating plant-based therapies is also emphasised.

Keywords: Anti-inflammatory drugs, animal model, drug screening model, *Escherichia coli*-induced inflammation, macrophage migration.

¹Department of Biological Sciences, Faculty of Applied Sciences, Rajarata University of Sri Lanka, Mihintale, Sri Lanka

²Department of Bioprocess Technology, Faculty of Technology, Rajarata University of Sri Lanka, Mihintale, Sri Lanka

Address for correspondence: Prof S. Hettiarachi, Department of Biological Sciences, Faculty of Applied Sciences, Rajarata University of Sri Lanka, Mihintale, Sri Lanka; Telephone: 0771101501 Email: sanath@as.rjt.ac.lk

 <https://orcid.org/0000-0002-8468-431X>