Biocatalysis and Agricultural Biotechnology 21 (2019) 101278

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab

Short communication

Toxicity and anti-inflammatory study of *Parmotrema austrosinense* extract against oxozalone induced intestinal inflammation in zebrafish (*Danio rerio*) model

Shanmugam Poornima[a], Nagarajan Nagarjun[a], Ponnusamy Ponmurugan[b,*], Balasubramanian Mythili Gnanamangai[a], Selvaraj Narasimman[a]

[a] Department of Biotechnology, K.S. Rangasamy College of Technology, Tiruchengode, 637 215, Namakkal District, Tamil Nadu, India
[b] Department of Botany, Bharathiar University, Coimbatore, 641 046, Tamil Nadu, India

ARTICLE INFO

Keywords:
Parmotrema austrosinense
Anti-inflammatory activity
toxicity
zebrafish model
histopathology

ABSTRACT

Lichens are symbiotic organisms producing unique secondary compounds having rich pharmacological value. The aim of the present study is to evaluate the anti-inflammatory potential of lichen *Parmotrema austrosinense*. Acetone extract of *P. austrosinense* was evaluated for its anti-inflammatory potential in zebrafish model. *In vitro* study evidenced the anti-inflammatory activity with inhibitory concentration of 67.43 and 64.68 µg/ml via protease inhibition assay and protein denaturation assay respectively. Toxicity study served as a supporting evidence which revealed that acetone extract of *P. austrosinense* has no toxicity against tested zebra fishes up to 600 µg/ml and considerable toxicity was observed at 800 µg/ml and 1000 µg/ml. Hence, 200, 400 and 600 µg/ml of acetone extract were used for the treatment of inflammation in fishes. *In vivo* study evidenced that 70% of the inflammation was cured by 600 µg/ml of acetone extract which in turn confirmed by histopathological evaluation of the intestinal tissues.

1. Introduction

Lichens are symbiotic combination of algae and fungi (Joel and Martin, 2005). Lichen secondary metabolites are very unique in nature which revealed varied biological activities. A single compound may exhibit numerous biological activities such as antimicrobial, antioxidant, anti-inflammatory, antidiabetic, anticancerous activities etc., Thus, these secondary metabolites find wide range of biomedical applications (Molnar and Farkas, 2010). Eastern Ghats is one of the lichen diversity hot spots in India, lichen diversity is observed at the altitude of above 500 MSL (Ponmurugan et al., 2016). The hill predominantly contains *Parmotrema* species. *P. austrosinense*, a terrestrial foliose lichen was chosen for the study, which contains lecanoric acid as the major secondary metabolite accumulates in the medulla region, where as atranorin and chloratronorin are found in upper cortex in very lesser quantity. Depsides and depsidones are the important lichen metabolites exhibiting various bioactivities (Ingolfsdotiir et al., 1996). Lecanoric acid and atranorin are the depsides present in *P. austrosinense* lichens reportedly having multifarious biological potential.

Inflammation is an essential defense mechanism which protects the body tissue from injury. The long-term use of non-steroidal anti-inflammatory drugs cause adverse side effects including gastrointestinal ulceration, cardiovascular and renal disorders (Horl, 2010). Plant based anti-inflammatory drugs drag more attraction now-a-days, since they are less toxic and safe too. Lichen secondary metabolites effectively inhibits leukotriene B4 biosynthesis which leads to anti-inflammatory effect (Kumar and Müller, 1999).

Zebrafish (*Danio rerio*) is a powerful experimental vertebrate model for anti-inflammatory study especially in developmental, genetic and toxicological evaluation (Brugman and Nieuwenhuis, 2017;Brugman, 2016). Well conserved gene, organ functions and immune system are observed between zebrafish and higher vertebrates (Sivamani et al., 2014; Quezada et al., 2013; Chen et al., 2013). Hence, zebra fish is chosen as an effective model for testing anti-inflammatory effect in this study. The present study aims to evaluate the anti-inflammatory activity of *P. austrosinense* extract. Since anti-inflammatory study was infrequently reported and little stated in lichen research, the present study attempted to examine *in vivo* anti-inflammatory potential of lichen extract for oxozalone induced inflammation in zebra fish model.

* Corresponding author.
E-mail address: drponmurugan@gmail.com (P. Ponmurugan).

https://doi.org/10.1016/j.bcab.2019.101278
Received 2 July 2019; Received in revised form 27 July 2019; Accepted 2 August 2019
Available online 12 August 2019
1878-8181/

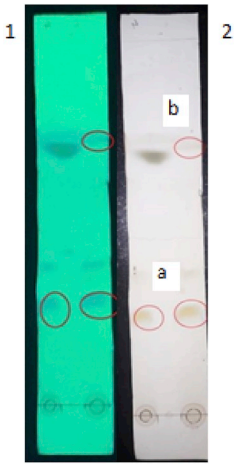

Fig. 1. TLC chromatogram representing lichen metabolites under 1) UV-radiation and 2) sulphuric acid spray a) Lecanoric acid b) atranorin.

2. Results and discussion

Lichen was collected from Yercaud hills, Eastern Ghats of Tamil Nadu, India. Further it was identified as *P. austrosinense* and authenticated by National Botanical Research Institute, Lucknow, Uttar Pradesh, India. The bioactive compounds present in the acetone extract was identified using Thin layer chromatography. Fig. 1 Represents the developed chromatogram using Toluene: Dioxane: Acetic acid (36:9:1) solvent system under UV- radiation and Sulphuric acid spray. Acetone is the promising solvent used for the extraction of lichen secondary metabolites such as lecanoric acid, atranorin etc., (Huneck and Yoshimura, 1996; Studzińska et al., 2008). Thin layer chromatogram of acetone extract of *P. austrosinense* confirmed the presence of secondary metabolites in the lichen sample such as atranorin and lecanoric acid.

Anti-inflammatory activity of acetone extract was evaluated by in vitro assays including protease inhibition assay and protein denaturation assay. The acetone extract of *P. austrosinense* have shown maximum inhibition at the concentration of 75 µg/ml which shows the inhibition of 63.5% (Fig. 2). The result revealed dose dependent activity of acetone extract of *P. austrosinense* (Bugni et al., 2009). The inhibition exhibited by acetone extract of *P. austrosinense* by protein denaturation assay is shown in Fig. 2. The extract exhibited maximum inhibition of 62.5% at the concentration of 75 µg/ml. IC_{50} value of *P. austrosinense* in protein denaturation assay is 67.43 µg/ml which is comparable with the IC_{50} value obtained by protease inhibition assay of about 64.68 µg/ml.

In vivo study was conducted in zebra fishes over the period of twenty-five days. The acclimatized fishes were subjected to

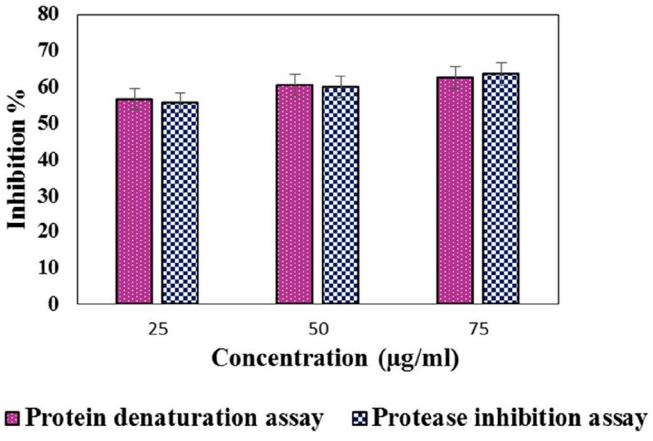

Fig. 2. *In vitro* antiinflammatory activity representing percentage inhibition.

Fig. 3. Anesthetization of zebrafish.

Fig. 4. Induction of inflammation via Intra-rectal mode in zebrafish.

toxicological evaluation and the anti-inflammatory study. The process of anesthetizing and induction of inflammation via intra-rectal mode by lichen extract and oxazolone is shown in Fig. 3 and Fig. 4. Acetone extract of *P. austrosinense* was tested in zebra fishes, maximum mortality rate was observed at 1000 mg/ml. Hence the concentration was decreased to microgram levels. With this reduced concentration, it was observed that at 200, 400 and 600 µg/ml all the fishes were survived (Table 1). Hence the anti-inflammatory study was further carried out with these three optimal dosage levels. The toxicology study revealed that *P. austrosinense* do not show any toxicity against the tested zebra fishes (Sroka and Dubino, 2018). The lichen secondary metabolites such as lecanoric acid and atranorin are responsible for the bioactive nature of the lichen *P. austrosinense* (Studzińska et al., 2008).

The microscopic observation of histopathology evaluation are shown in Fig. 5. The microscopic observation of positive control shows

Table 1
Dose optimization of lichen extract for toxicity evaluation in zebra fish model.

Concentration of lichen extract	After incubation of ten days			
	Cycle – 1 (mg/ml)		Cycle – 2 (µg/ml)	
	Alive	Dead	Alive	Dead
200	80.00 ± 0.00	20.00 ± 0.00	100.00 ± 0.00	–
400	73.33 ± 6.67	26.67 ± 6.67	100.00 ± 0.00	–
600	73.33 ± 6.67	26.67 ± 6.67	100.00 ± 0.00	–
800	53.33 ± 6.67	46.67 ± 6.67	80.00 ± 0.00	20.00 ± 0.00
1000	46.67 ± 6.67	53.33 ± 6.67	60.00 ± 0.00	40.00 ± 0.00

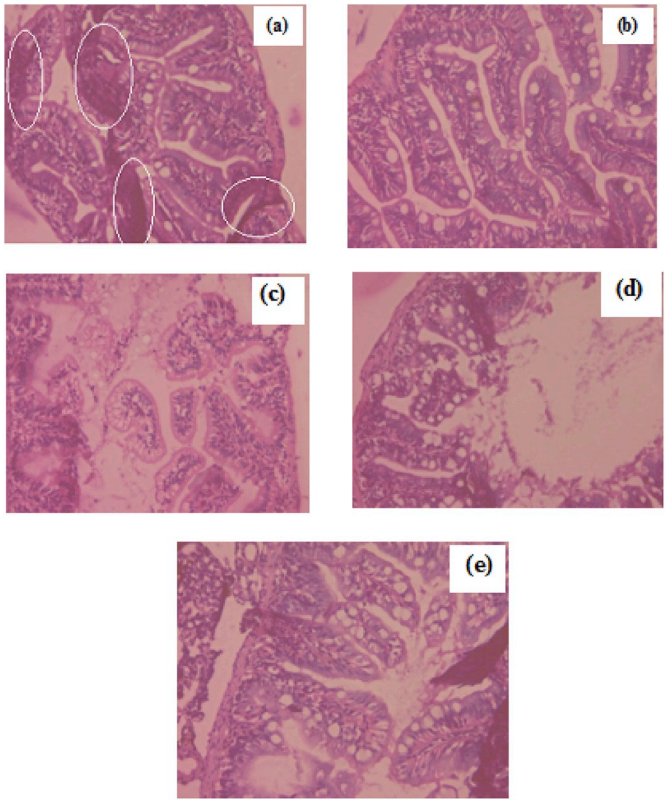

Fig. 5. Microscopic view of (a) inflammated zebrafish intestine (positive control), (b) healthy intestine (negative control), (c) treatment with 600 µg/ml, (d) treatment with 400 µg/ml, (e) treatment with 200 µg/ml of lichen extract.

the formation of the inflammation in the zebrafish intestine (Fig. 5a). The inflammation induced by the standard drug oxazalone is confirmed by the thickend cell wall of the intestine. In case of the negative control, the microscopic observation indicated the undisturbed intestine. The cell wall of the intestine was found to be thin without any inflammation (Fig. 5b). The treatment of 200 µg/ml of acetone extract of *P. austrosinense* exhibited 20% cure of the inflammation but still the walls of the intestine was observed with inflammation (Fig. 5c). At 400 µg/ml, about 50% of the inflammation was cured (Fig. 5d), when compared to 200 µg/ml, the walls of the intestine became thin. At 600 µg/ml, about 70% of the inflammation has been cured (Fig. 5e). When comparing to other two concentrations, 600 µg/ml have shown the best results with maximum healing ability.

3. Conclusion

In vitro and *in vivo* studies evidenced that acetone extract of *P. austrosinense* exhibited significant anti-inflammatory activity. The bioactive nature of *P. austrosinense* may be attributed due to the presence of the secondary compounds such as lecanoric acid and atranorin which in turn confirmed by thin layer chromatography. The present study suggests that *P. austrosinense* can act as a promising source for the treatment of inflammation-based disorders and further recommends that evaluating diversified biological potential of secondary metabolites will facilitate commercialization of such compounds for various biomedical applications.

Conflicts of interest

The authors declare that there is no conflict of interest in this study.

Acknowledgement

The authors are grateful to the Management and Principal of K.S.Rangasamy College of Technology, Tiruchengode, Namakkal District, Tamil Nadu, India and also gratefully acknowledge DST FIST (Fund for Infrastructure for Science and Technology) FIST No: 368 (SR/FST/College – 235/2014)] and DBT-STAR College Scheme (BT/HRD/11/09/2018) for their support for the instrumentation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101278.

References

Brugman, S., 2016. The zebrafish as a model to study intestinal inflammation. Dev. Comp. Immunol. 64. https://doi.org/10.1016/j.dci.2016.02.020.

Brugman, S., Nieuwenhuis, E.E.S., 2017. Oxazolone-induced intestinal inflammation in adult zebra fish, inflammation: methods and protocols. Methods Mol. Biol. 1559, 311–318.

Bugni, T.S., Andjelic, C.D., Pole, A.R., Rai, P., 2009. Ireland CM, Barrows LR. Biologically active components of a Papua New Guinea analgesic and anti-in flammatory lichen preparation. Fitoterapia 80 (5), 270–273. https://doi.org/10.1016/j.fitote.2009.03.003.

Chen, Y.H., Wang, W.H., Wang, Y.H., Lin, Z.Y., Wen, C.C., Chern, C.Y., 2013. Evaluation of the anti-inflammatory effect of chalcone and chalcone analogues in a zebrafish model. Molecules 18, 2052–2060.

Horl, W.H., 2010. Nonsteroidal anti-inflammatory drugs and the kidney. Pharmaceuticals 3 (7), 2291–2321. https://doi.org/10.3390/ph3072291.

Huneck, S., Yoshimura, I., 1996. Identification of Lichen Substances. Springer-Verlag, Berlin, Heidelberg.

Ingolfsdotiir, K., Gissurarson, S.R., Moller-Jakic, B., Breu, W., Wagner, H., 1996. Inhibitory effects of the lichen metabolite lobaric acid on arachidonate metabolism in vitro. Phytomedicine 2 (3), 243–246.

Joel, B., Martin, G., 2005. Lichens-a promising source of bioactive secondary metabolites. Plant Genet. Resour. 3 (2), 273–287. https://doi.org/10.1079/PGR200572.
Kumar, S., Müller, K., 1999. Lichen metabolites. 1. Inhibitory action against leukotriene B4. Biosynthesis by a non-redox mechanism. J. Nat. Prod. 62 (6), 817–820.
Molnar, K., Farkas, E., 2010. Current results on biological activities of lichen secondary metabolites. J. Phys. Sci. 65, 157–173.
Ponmurugan, P., Ayyappadasan, G., Verma, R.S., Nayaka, S., 2016. Survey, distribution pattern and elemental composition of lichens in Yercaud hills of Eastern Ghats in southern India. J. Environ. Biol. 37, 407–412.
Quezada, M., Alvarez, M., Peña, O.A., Henríquez, S., Alençon, C.A., Lange, S., Oliva, B., Owen, G.I., Allende, M.L., 2013. Antiangiogenic, antimigratory and antiinflammatory effects of 2-methoxyestradiol in zebrafish larvae. Comp. Biochem. Physiol., C 157, 141–149.
Sivamani, S., Joseph, B., Kar, B., 2014. Anti-inflammatory activity of Withania somnifera leaf extract in stainless steel implant induced inflammation in adult zebrafish. J. Genet. Eng. Biotechnol. 12, 1–6.
Sroka, E.S., Dubino, A., 2018. Lichens as a source of chemical compounds with anti-inflammatory activity. Herba Pol. 64 (1), 56–64. https://doi.org/10.2478/hepo-2018-0005.
Studzińska, E., Witkowska-Banaszczak, E., Bylka, W., 2008. Związki biologicznie czynne porostów. Herba Pol. 54 (1), 79–88 2008.