Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

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

Document heading

Anti-inflammatory activity of the glandular extracts of *Thunnus alalunga*

A.K. Azeem*, C Dilip, S S Prasanth, V Junise Hanan Shahima, Kumsr Sajeev, C. Naseera

Al Shifa College of Pharmacy, Poonthavanam P.O, Kizhattur, Perinthalmanna, Malappuram dist, Kerala,679329, India

ARTICLE INFO

Article history:
Received 12 July 2010
Received in revised form 27 July 2010
Accepted 7 September 2010
Available online 20 October 2010

Keywords:
Thunnus alalunga
Anti-inflamattory
HRBC
Carrageenan

ABSTRACT

Objective: To evaluate the anti-inflamattory activity of *Thunnus alalunga* by both *in vitro* and *in vivo* methods. **Methods:** Anti-inflammatory activity of the chloroform water extract of *Thunnus alalunga* was done by both in vitro and *in vivo* methods. *In vitro* method was done by human red blood cells membrane stabilization method (HRBC). *In vivo* evaluation was estimated on Wister albino rats. Acute toxicity studies were done on the extract and no toxicity was reported. **Results:** The percentage protection exhibited by 300 mg/mL concentration was more when compared to the other ones. The 400 mg/mL concentration showed potent activity on comparison with the standard during in vivo evaluation. **Conclusions:** In both means of estimation the extract of *Thunnus alalunga* was found to possess significant anti-inflammatory activity.

1. Introduction

Inflammation is a reaction of living tissues towards injury, it comprises systemic response and local responses[1]. The ocean and sea occupies nearly 70% of the world. The marine flora and fauna are rich sources of biologically active compounds. A large number of toxic metabolites have been isolated and characterized from protozoan, sponge, coelenterate, echinoderms, mollusks, sea snakes and fishes. The great interest in these chemically varied compounds has resulted in the development of novel therapeutic agents for the treatment of various diseases afflicted by human beings. Marine species studied in the recent years have yielded a variety of compounds which possess known or novel pharmacological activities in mammels and have exhibited antimicrobial, anti-inflammatory and antineoplastic property. *Thunnus alalaunga* of the family Scrombidae comes under the order Perciformes and class Actinopterygii was commonly known as *Albacore tuna*. It lives in the tropical and subtropical region with medium resilience, of minimum population with a doubling time of 1.4–4.4 yrs. The fish folk community of Southern part of India was using the steamed and baked intestine of this fish for the treatment of severe back and neck pains. The present study aims to authentify that traditional information by both *in vitro* and *in vivo* anti-inflammatory screening.

*Corresponding author: A.K. Azeem, Asst. Professor, Al Shifa College of Pharmacy, Poonthavanam P.O, Kizhattur, Perinthalmanna, Malappuram dist, Kerala, 679329, India.
Tel: +919447005783
E-mail: akazeem80@gmail.com

2. Materials and methods

2.1. Preparation of extracts

Fresh tuna was collected from Ponnani of Malappuram district Kerala and was authenticated by fisheries experts. The glands of fish was separated and ground to a coarse form. It was then washed thoroughly with distilled water and dried carefully with the help of filter paper. Then it was extracted with a mixture of chloroform and water using a rotary shaker. The extract was concentrated under reduced pressure and preserved at low temperature.

2.2. Chemicals and instruments

All chemicals used in the estimation were of analytical grade. Carrageenan was purchased from sigma chemicals. Reference standard diclofenac sodium was obtained as gift sample from MRL labs Chennai. Shimadzu 1701 UV Visible spectrophotometer was used for the *in vitro* study.

2.3. Animals

Adult Wister albino rats (60–120 g) of either sex were

used for the *in vivo* evaluation. They were housed under standard laboratory conditions and were fed with standard animal feed and water *ad libitum*. The experimental protocol was approved by institutional animal ethical committee.

2.4. Acute toxicity test

Acute toxicity study was performed as per Organization of Economic Cooperation and Development guidelines 423[2]. (Acute toxicity class method).

2.5. Anti-inflammatory activity (in vitro)

Human red blood cell membrane stabilisation method (HRBC method)[3] was used for the estimation of anti-inflammatory activity *in vitro*. Blood was collected from healthy volunteers and it was mixed with equal volume of sterilized Alsevers solution. This blood solution was centrifuged at 3 000 rpm and the packed cells were separated. The packed cells were washed with isosaline solution and a 10% v/v suspension was made with isosaline. This HRBC suspension was used for the estimation of anti inflammatory property. Different concentrations of extract, reference sample and control were separately mixed with 1 mL of phosphate buffer, 2 mL of hyposaline and 0.5 mL of HRBC suspension. All the assay mixtures were incubated at 37 ℃ for 30 min and centrifuged at 3 000 rpm. The supernatant liquid was decanted and the hemoglobin content was estimated by a spectrophotometer at 560 nm. The percentage hemolysis was estimated by assuming the hemolysis produced in the control as 100%.

Percentage protection= [1- (OD sample/ OD control)] × 100%

2.6. Anti-inflammatory activity (in vivo)

Paw oedema was induced on each rat by injecting 0.1 mL of carrageenan on physiological saline to the left hind paw. The extracts at different concentrations were administered orally 30 min prior to Carrageenan administration[4]. Paw volumes were measured at 60 min, 120 min, 180 min and 240 min by mercury displacement method using plethysmograph. The percentage inhibition of paw volume in extract treated groups was compared with control. Diclofenac sodium (5 mg/kg) was used as the standard.

2.7. Statistical analysis

Statistical analysis was done using one way analysis of variance followed by Dunnets *t* test. *P* values < 0.05 were considered significantly.

3. Results

3.1. Acute toxicity studies

The extracts of *Thunnus alalunga* did not cause any sign of toxicity up to 2 000 mg/kg body weight and hence it was considered to be safe.

3.2. Anti-inflammatory activity (in vitro)

Thunnus alalunga glandular extracts at different concentrations (100 mg/mL, 200 mg/mL, 300 mg/mL, 400 mg/mL) showed significant stabilization towards HRBC membranes. The percentage protection at concentration 300 mg/mL is more when compared to the other concentrations. However on higher concentration the percentage protection was found to be decreased. The results were tabulated in Table 1.

Table 1

In vitro anti-inflammatory activity of glandular extracts of *Thunnus alalunga* by HRBC method.

Concentration(mg/mL)		Percentage protection(%)
Thunnus alalunga	100	22.35
	200	29.86
	300	31.49
	400	28.86
Diclofenac sodium	5	32.55

Percentage protection is the mean triplicate readings.

3.3. Anti- inflammatory activity (in vivo)

The extracts of *Thunnus alalunga* at different concentrations showed significant reduction in the paw volume of rats. The extract at concentration of 400 mg/mL showed potent activity, comparing with the reference standard Diclofenac sodium. The results were tabulated in Table 2.

4. Discussion

Inflammation is common phenomenon and it is a reaction of living tissues towards injury. Steroidal anti-inflammatory agents will lyse and possibly induce the redistribution of lymphocytes causing rapid and transient decrease in peripheral blood lymphocyte counts to effect longer term response. *Thunnus alalunga* of the family Scrombidae was a common edible fish of Kerala coast and it is popularly known as tuna. Chemical evaluation of the glandular extract of *Thunnus alalunga* reveals the presence of steroids. Here anti-inflammatory activity was performed based on the folk lore information using two methods. HRBC method was selected for the *in vitro* evaluation of anti-inflammatory property because the erythrocyte membrane is analogous to the lysosomal membrane[5] and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release. The result indicted that the glandular extract of *Thunnus alalunga* at various concentrations had significant anti-inflammatory property. Carrageenan induced inflammation is a useful model for the estimation

Table 2
Anti-inflammatory activity by Carrageenan induced oedema (mean±SEM)(%) (n=6)

Drug	Dose (mg/kg)	Carrageenan induced oedema (Volume in mL)			
		60 min	120 min	180 min	240 min
Control		0.370±0.020	0.390±0.010	0.430±0.020	0.430±0.010
Diclofenac	5	0.170±0.010(59.3) *	0.160±0.020(65.4) *	0.140±0.004*(66.7)	0.110±0.010*(77.05)
Extract	100	0.280±0.003(32.5) *	0.160±0.020(34.7) *	0.200±0.010*(51.3)	0.180±0.004*(55.8)
Extract	200	0.230±0.004(43.3) *	0.240±0.010(49.6) *	0.190±0.020*(53.7)	0.140±0.007*(64.8)
Extract	300	0.270±0.009(48.5) *	0.230±0.010(49.1) *	0.260±0.020*(65.5)	0.240±0.020*(68.2)
Extract	400	0.160±0.010(57.4) *	0.250±0.004(65.9) *	0.130±0.020*(67.7)	0.110±0.009*(78.6)

*P<0.001 with control.

anti inflammatory effect. The development of oedima in the paw of the rat after the injection of Carrageenan is due to the release of histamine, serotonin and prostaglandin like substances[6-8]. Glandular extract of *Thunnus alalunga* showed significant anti inflamatory activity. This significant anti-inflammatory effect may be due to the inhibition of any inflammatory mediators by the steroids[9] present in the extract. The present result indicates the efficacy of *Thunnus alalunga* as an effective therapeutic agent in the treatment of acute inflammatory conditions. The result of present study authentifies the folk lore information on the anti-inflammatory property of the glandular extract of *Thunnus alalunga*. Further detailed study is in process for the isolation of active constituent responsible for this property and to idendify the possible mechanism of its anti inflamattory property.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors are thankful to the management, Director and faculties of Alshifa College of Pharmacy Perinthalmanna for rendering the necessary requirements for this work.

References

[1] Hardmann JA, Limbard LE, Goodmann A. *Pharmacological basis therapeutics*. US: Mc Graw-Hill Inc.; 1998, p. 1465.
[2] Ecobichon DJ. *The basis of toxicology testing*. New York: CRC Press;1997, p. 43-86.
[3] Gandhidasan R, Thamaraichelvan A, Baburaj. Antiinflamattory action of *Lanea coromondelica* by HRBC membarane stabilisation. *Fitotherapia* 1991; **62**: 82-3.
[4] Chou CT. The anti-inflammatory effect of *Tripterygium wilfordii* Hook F on adjuvant-induced paw edema in rats and inflammatory mediators release. *Phytother Res* 1997; **11**: 152-4.
[5] Vadivu R, Lakshmi KS. *In vitro* and *in vivo* anti-inflammatory activity of leaves of *Symplocos cochinchnensis* (Lour) *Moore* ssp laurina. *Bangladesh J Pharmacol* 2008; **3**: 121-4.
[6] Ilavarasan R, Mallika M, Venkataraman S. Anti-inflammatory and antioxidant activities of *Cassia fistula* Linn bark extracts. *Afr J Trad CAM* 2005; **2** (1): 70-85.
[7] Gupta GD, Gaud RS. Antiinflammatory activity of tenoxicam gel on carrageenan-induced paw oedema in rats. *Indian J Pharmaceutical Sciences* 2005; **68**(3): 356-9.
[8] Brahmbhatt MR, Patel JM, Patel VB, Saluja AK. Analgesic and antiinflammatory activity of leaves of *Rivea hypocrateriformis*. *J Pharmacognosy & Phytotherapy* 2010; **1**(1): 1-3.
[9] Harbone JB. *Phytochemical methods, a guide to modern technique of plant analysis*. New York: Chapman and Hall; 2007, p. 4-8.