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Research Article

Extraction and Immunomodulatory Screening of Fish Oil on *Cyprinus carpio.*

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ABSTRACT

The proposed work describe the extraction of oil from fish species. Fish species used for oil extraction *Cyprinus carpio.* The Asst. Commissioner of Fishery, Government Department Of Fishery Office Sangli Authentificated the fish species. Type of free fatty acid present differs to great extent from species to species or according to its water environment. For extraction of fish oil methanol & chloroform in ratio (2:1) was used. For isolation of active phytochemical constituent from fish oil done by using cyclohexane, methanol, chloroform in ratio (5:2:4) mobile system. Immunomodulatory activity perform on swiss albino mice In immunomodulatory activity, it shows that Delayed Type Hypersensitivity (DTH) response in terms of increase in the mean difference of paw thickness in mm, compared with vehicle control & negative control. Heightened delayed type hypersensitivity reaction suggest activation of cellular immune system.

Keywords: Fish oil, Delayed Type Hypersensitivity, Immunomodulation

INTRODUCTION

The fish oil contain different PUFA, which are brought about by modulation of the amount and types of eicosanoids made, and other effects are elicited by eicosanoid-independent mechanisms, including actions upon intracellular signaling pathways, transcription factor activity and gene expression. Fish oil have anti-inflammatory properties and, therefore, might be useful in the management of inflammatory and autoimmune diseases[10]. For extraction fish oil *Cyprinus carpio* species is used. The common carp *Cyprinus carpio* is a widespread freshwater fish of eutrophicwaters in lakes and large rivers in Europe and Asia. Body elongated and somewhat compressed. Lips thick. Two pairs of barbels at angle of mouth, shorter ones on the upper lip. Dorsal fin base long with 17-22 branched rays and a strong, toothed spine in front; dorsal fin outline concave anteriorly. Anal fin with 6-7 soft rays, posterior edge of 3rd dorsal and anal fin spines with sharp spinules. Lateral line with 32 to 38 scales. Pharyngeal teeth 5:5, teeth with flattened crowns. Colour variable, wild carp are brownish-green on the back and upper sides, shading to golden yellow ventrally. The objective of the work is to extract the fish oil from fish species *Cyprinus carpio* and To screen the Immunomodulatory activity.

MATERIALS AND METHODS

Materials

Chloroform, Methanol, Butylated hydroxytoluene (BHT), Sodium sulphate anhydrous

Methods[1,2,3]

Fish tissue (50g) were homogenized in a blender for 2 minutes with a mixture of methanol (100 ml) and chloroform (50 ml). Then 50 ml of chloroform was added to the mixture. After blending for an additional 30 seconds, distilled water (50 ml) was added. The homogenate was stirred with a glass rod and filtered through a Whatman no.1 filter paper on a Buchner funnel under vacuum suction. 20 ml chloroform was used to rinse the remainder. The filtrate was allowed to settle to separate into the organic and aqueous layers. The chloroform layer containing the oil was transferred into another beaker and 3 g of anhydrous sodium sulphate was added to remove any remaining water. The mixture was filtered through a Whatman no. 1 filter paper and chloroform was used to rinse the remainder. Finally, 0.02 g of BHT was added to the oil as an antioxidant. The solution was then evaporated at room temperature. After extraction of oil then isolate the active phytochemical constituent from fish oil done by using cyclohexane, methanol, chloroform in ratio (5:2:4) mobile system. The isolated phytochemical constituent used to screening immunomodulatory activity.

Screening Method[8,9]

Drug solution

1. levamisole (50 mg/kg)
2. cyclophosphamide (100 mg/kg)

Animals used

1. Species : swiss albino mice
2. Age/ weight: 4-6 week (20-25g.)

Cell mediated Immune Response; Delayed Type Hypersensitivity (DTH)

Cellular Immune Response

Foot pad edema in mice was used for detection of cellular

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Table 1: Cell mediated Immune Response

Sr. No.	Groups	Treatment , Dose & Rout	Treatment Scheduled
1	Control	Distilled water, 10 ml/kg, (p.o.)	1st to 21st day
2	Negative control	Cyclophosphamide (cyp) 100 gm/kg (p.o)	Single dose on 14th day ; two hr. after immunization with sheep RBC.
3	Oil treated	71.43 mg/kg(p.o)	1st to 21st day
4	Oil + cyp. treated	71.43 mg/kg(p.o) + cyp. 100 mg/kg(p.o.)	Oil 1st to 21st day+ cyp. As a single dose on 14th day two hr. after immunization with SRBC.
5	LMS treated	50mg/kg (p.o.)	Levamisole from 1st to 21st day, cyp. As a single dose on 14th day two hr. after immunization
6	LMS + cyp. treated	50mg/kg(p.o.) +cyp. 100mg/kg	Levamisole from 1st to 21st day,+ cyp. As a single dose on 14th day two hr. after immunization

Cyp.- cyclophosphamide; LMS- levamisole

Table 3: Effect Of Oil On Cellular Immunity In Delayed Type Hypersensitivity Induced Footpad Edema

S.No.	Groups(n=5)	Treatment , Dose & Rout	Mean diff. of paw edema in (mm)
1	Control	Distilled water, 10 ml/kg, (p.o)	0.203±0.01
2	Negative control	Cyclophosphamide (cyp) 100 gm/kg (p.o)	0.440 ±0.007
3	Oil treated	71.43 mg/kg(p.o)	0.894±0.001
4	Oil + cyp. treated	71.43 mg/kg(p.o)+ cyp. 100 mg/kg(p.o.)	0.953±0.001
5	LMS treated	50mg/kg (p.o.)	0.616±0.08
6	LMS + cyp. treaed	50mg/kg (p.o.)+ cyp. 100 mg/kg(p.o.)	0.960±0.001

Table 2: Formula of Alsevere's solution

Chemicals	Quantity (g/l)
Sodium chloride	4.2
Sodium citrate	8.0
Citric acid anhydrous	0.55
Glucose	20.5
Distilled water	1000ml

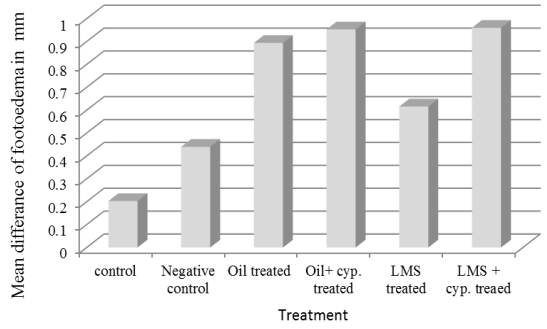

Figure 1: Delayed Type Hypersensitivity (DTH) Response on Swiss albino mice

immune response. on 21st day , injection of sheep RBCs (0.1ml of 20% SRBCs) in the sub-plantar region of right hind paw in the volume of 0.03 ml and normal saline in left hind paw in same volume. Foot pad reaction was assessed after 24 hr. i.e. on 22nd day, in terms of increase in the thickness of the right hind footpad was measured. The footpad reaction was expressed as the difference in the thickness (mm) between the right foot pad injected with SRBCs and left foot pad injected with normal saline.

Preparation of sheep RBC (SRBCs)

Sheep blood was collected in sterile Alsevere's solution in 1:1 proportion of Alsevere's solution (freshly prepared). Blood was kept in the refrigerator & processed for preparation of sheep RBC batch, by centrifugation at 2000 rpm for 10 min. & washing with physiological saline 4-5 time & then suspending into buffered saline for further use.

Statistical analysis

The result are expressed as mean ± S.E.M. value of foot pad edema were analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. Value $p \leq 0.01$ were considered statistically significant.

RESULTS & DISCUSSION

In immunomodulatory activity, it shows that DTH response in terms of increase in the mean difference of paw thickness in mm, compared with vehicle control & negative control. Heightened delayed type hypersensitivity reaction suggest activation of cellular immune system.

DTH response is direct co-related to cell-mediated immunity and was significantly increased with fish oil in combination with cyclophosphamide. Intermediate tubers as compared to normal control. The significant fish oil increase in Immunomodulatory potential attributed due to presence of Essential Fatty Acids

CONCLUSION

From above experimental work fish oil shows that DTH response in terms of increase in the mean difference of paw thickness in mm, compared with vehicle control & negative control. Heightened delayed type hypersensitivity reaction suggest activation of cellular immune system.

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