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Anti - Hyperlipidemic Activity of Cuttle Fish Ink

[1]Su. Sangeetha*, [2]S. Karpagam Kumara Sundari,[3]K.A.S. Mohamed Shafeeq,[4]T. Ramesh

[1]*Student, Department of Pharmacology, Periyar College of Pharmaceutical Sciences, Tiruchirappalli, Tamilnadu.*
[2]*Professor, Head Department of Pharmacology, Periyar College of Pharmaceutical Sciences, Tiruchirappalli, Tamilnadu.*
[3]*Associate Professor, Department of Pharmacology, Periyar College of Pharmaceutical Sciences, Tiruchirappalli, Tamilnadu.*
[4]*Associate Professor, Department of Marine Science, CAS in Marine Biology, Annamalai University, Parangipettai, Tamilnadu.*
Corresponding Author: Su. Sangeetha

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ABSTRACT: The compounds are being isolated from the number of marine organisms virtually soared and now exceeds 10,000 with hundreds of new compounds still being discovered every year.Huge innovation opportunities exist in using an underutilized / waste / by-product to create new healthy products to consumers. Evaluation of anti-hyperlipidemic activity induced by Triton-x 100 in animal models using Cuttle Fish Ink extract at a dose of 200mg/kgp.o. Oral administration of cuttle fish ink produced significant reduction in serum lipid parameters such as Cholesterol, Triglycerides, Low Density Lipoprotein (LDL), Very Low Density Lipoprotein (VLDL) and increased in High Density Lipoprotein (HDL) in rats as compared to reference drug (Atorvastatin 10mg/kg, p.o.). Thus Cuttle Fish Ink possess anti-hyperlipidemic activity with no toxicity.

KEYWORDS: Marine Organisms, Cuttle Fish Ink, Triton X 100, Anti-hyperlipidemic

I INTRODUCTION

In recent years, great attention towards the potential pharmacological utilization of bioactive natural products.[1] Mollusca is the 2nd largest phylum in the marine environment around 1, 00,000 species found in all over the world.[2] Huge innovations were obtained from the waste by product of the marine sources to discover the new compounds to consumers.Approximately 5000 species of sponges, 11000 species of corals, jellyfish and sea anemones, 900 species of segmented worms, 1,00,000 species of annelids, polychates, snails, clams and octopus, 6000 species of sea stars and sea cucumber and 200 species of a sea squids are present in the marine environment.[3] The Cuttle fish around 304 species in the world that belongs to the familyloliginidae. The cuttle fish contains two glands one is producing mucus and other one is ink sac, with its ink gland produces a black ink containing melanin. Cuttle fish are beneficial to human kind, the ink shows potential anti-retroviral activity, the peptidoglycan and acetone delipidated ink of cuttle fish shows antitumor activity. Cuttle fish ink has wide application in homeopathic medicine, in food products as antiseptic, antibacterial drug. [4]

Hyperlipoproteinaemias (HPL) are conditions in which the concentration of cholesterol or triglyceride (TG) carrying lipoproteins in the plasma is elevated above normal, increase in lipoproteins can hasten the development of atherosclerosis and is a risk factor for myocardial infarction. [5]Lipids and proteins form complexes called lipoproteins and circulate in the blood vessels. There are 4 types of lipoproteins: i) Low density lipoproteins (LDL) ii) High density lipoproteins (HDL) iii) Very low density lipoproteins (VLDL) iv) Chylomicrons. LDL is the primary carrier of cholesterol while VLDL is of triglycerides. There are two different pathways such as endogenous and exogenous pathway for their transport; cholesterol and triglycerides absorbed from the gut are transported as chylomicrons. Chylomicrons are hydrolyzed by the action of lipoprotein lipase (LPL) and free fatty acids are released which are taken up by muscle and adipose tissue. [6]

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II MATERIALS AND METHODS

2.1 Extraction

The fresh Cuttle fish were purchased directly from a fishmonger from Muthupettai region of Nagapattinam and the fish was dissected to collect the Ink Sac and transferred immediately in ice box. The Ink was dissolved with equal volume of Phosphate Buffer Saline (pH – 7) andultrasonicated.Theresultant extract was centrifuged at 5000rpm for 10 minutes, and the supernatant liquid was stored at -20°C until its use.[7]

2.2 Preparation of Phosphate Buffer Saline

Disodium Hydrogen Phosphate – 2.38 g, Potassium Hydrogen Phosphate–0.19g, Sodium Chloride–0.8g is taken and dissolved in distilled water and made upto 1000ml. Finally pH is adjusted at 7

2.3 Animal Experimentation

Antihyperlipidemic activity of Cuttle Fish Ink extract was carried out in the Department of Pharmacology, Periyar College of Pharmaceutical Sciences, Tiruchirappalli, Tamilnadu, India. Animal facility of this Institute is approved by CPCSEA. The experimental protocols for the Antihyperlipidemic activities have been approved by the Institutional Animal Ethics Committee and conducted according to the guidelines of Indian National Sciences Academy for the use and care of experimental animals. IAEC approved this approval number PCP/IAEC/003/2019. The animals were maintained at a well-ventilated temperature controlled 30°c ±1°c animal room for 7 days prior to the experimental period food and waterad libitum. The animals were acclimatized to laboratory condition before the test, each animal was used only once.

2.4Triton X 100 Induced Hyperlipidemia [8, 9]

Male Wistar albino rats weighing 200-250g, used for the study were fed on pellet diet and water ad libitum. Hyperlipidemia was induced by intraperitoneal administration of Triton X 100 at a dose of 100mg/kg.

2.5 Experimental Design

Rats were divided into 4 groups containing 6 animals in each group

Group I : Normal diet water ad libitum

Group II : Normal diet and Triton X 100 (100mg/kg) (positive control)

Group III : Normal diet and Triton X 100 (100mg/kg) + Cuttle fish Ink extract (200mg/kg, p.o.)

Group IV : Normal diet and Triton X 100 (100mg/kg) + Atorvastatin (10mg/kg, p.o.)

2.6 Biochemical Parameters

Animals were fasted overnight upto 18hrs hyperlipidemia was induced by single intraperitoneal injection of freshly prepared solution of Triton X-100 (100 mg/ kg) in normal saline. After 72 hours reference drug (Atrovastatin 10mg/kg, p.o) and test extract (Cuttle fish ink 200mg/kg, p.o.) were administered to the respective groups for 7 days.

2.7 Statistical Analysis

The results are expressed as Mean ± SEM (n=6) two way ANOVA using a Graphpad and PRISM software version 7.2.1. **** P<0.0001 were considered as statistically significant.

III RESULTS AND DISCUSSION

Intraperitoneal administration of Triton X 100 (100mg/kg) increased the level of serum total cholesterol, triglycerides, LDL, VLDL and decrease in the level of good cholesterol carrier HDL by inhibiting the lipase activity.[10] Elevated level of blood cholesterol especially LDL was the major risk factor for the coronary heart disease and HDL as cardio protective protein. Treatment with Cuttle Fish Ink extract (200 mg/kg, p.o) significantly decreased the level of cholesterol, triglycerides, VLDL and LDL as compared to hyperlipidemic control. There was significant increase in HDL as compared to control.

Treatment with Cuttle Fish Ink extract (200 mg/kg, p.o) significantly decreased the level of cholesterol, triglycerides, VLDL, LDL and increased in HDL as compared to the reference drug (Atrovastatin 10mg/kg, p.o). Hypolipidemic activity may be due to the presence of flavonoids, amino acids, alkaloids in the Cuttle fish ink, it may inhibit the cholesterol biosynthesis and modify the lipogenic and lipolytic enzymes due to reduced lipid metabolism.[11] It may causes the decrease in triglyceride level by stimulation of lipoprotein lipase activity and reduces the serum cholesterol level may be inhibition of HMG-CoA reductase activity. [12]

The body weight of the animal was measured initially on 1st day & 8thday(Table 1). The blood was collected at the end of 8th day by cardiac puncture under mild anesthesia, the serum was

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assayed for total cholesterol, total triglycerides, HDL-C, LDL-C and VLDL-C. (Table 2)

Table 1: Body weight changes in Antihyperlipidemic activity of Cuttle fish Ink

S.No.	Group	Body Weight (g)	
		Initial 1st day	At the end of study 8th day
1.	Control (Saline 2ml/kg, p.o.)	210.29 ± 0.41	211.53 ±0.16
2.	Lipid Control (Triton-X 100, 100mg/kg, i.p)	211.22 ± 0.34	226.42 ± 0.14
3.	Standard drug (Atorvastatin10mg/kg,p.o.)	209.84 ± 0.26****	221.33 ± 0.35****
4.	Test extract (Cuttle fish Ink 200mg/kg, p.o.)	210.78 ± 0.22****	220.78 ± 0.22****

n = 6 Values are expressed as ± S.E.M. Where, **** P < 0.0001 vs Control by two way ANOVA

Table 2: Antihyperlipidemic activity of Cuttle Fish Ink

S. No.	Groups	Serum Lipid Profile (mg/dl)				
		Total Cholesterol	Total Triglycerides	HDL	LDL	VLDL
1	Control (Normal Saline 2ml/kg, p.o.)	49.05 ± 0.16	47.98 ± 0.67	21.68 ± 0.41	16.79 ± 0.16	11.04 ± 0.17
2	Lipid Control (Triton X 100 100mg/kg, i.p)	219.27 ± 0.16	197.50 ± 0.27	12.98 ± 0.19	45.31 ± 0.11	32.54 ± 0.16
3	Standard drug (Atorvastatin 10mg/kg, p.o.)	88.32 ± 0.47 ****	97.01 ± 0.23****	15.14 ± 0.11****	13.00 ± 0.05****	13.40 ± 0.25****
4	Test extract (Cuttle Fish Ink 200mg/kg, p.o.)	161.76 ± 1.72 ****	106.08 ± 0.20 ****	17.29 ± 0.18****	21.81 ± 0.56****	10.75 ± 0.18****

n = 6 Values are expressed as ± S.E.M. Where, **** P< 0.0001 vs Control by two way ANOVA

IV CONCLUSION

Anti-hyperlipidemic activityof Cuttle Fish Ink extract (200mg/kg, p.o.) produces significant reduction in serum lipid parameters such as Cholesterol, Triglycerides, Low Density Lipoprotein (LDL), Very Low Density Lipoprotein (VLDL) and elevation in High Density Lipoprotein (HDL) in rats as compared to reference drug (Atorvastatin 10mg/kg, p.o.) treated group. Hence it is concluded as a better anti-hyperlipidemic agent.

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