Evaluation of hepatoprotective activity of the commerson’s anchovy (Stolephorus commersonnii)

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ABSTRACT

Objective: In the present study, we have chosen a fish Stolephorus commersonnii which is abundant in whitebait catches along Kerala coast. This fish is reported to contain higher content of essential amino acids, monounsaturated fatty acids, polyunsaturated fatty acids and minerals. The medicinal values of this species have not been reported and remain unexplored. Hence, there is a necessity to explore their uses and to conduct the pharmacological studies to ascertain their therapeutic properties. Based on literature search, no study has been carried out to scientifically validate the fish S. commersonnii possesses hepatoprotective property. Hence, this study was carried out to investigate the hepatoprotective effect of the S. commersonnii.

Materials and Methods: The fish was purchased from the fish market, Ernakulam, Kerala, India. The fish were kept on ice until processed in the lab. Fish is washed and cleaned properly. The tissue is homogenized and extracted with chloroform: methanol solvent system. The extract is transferred to a dark glass container and stored in a freezer. The preliminary analysis of fish extract was carried out by simple qualitative methods. The hepatoprotective activity of Stolephorus commersonnii (SC) extract at 300 mg/kg is carried out in isoniazid induced hepatotoxic model. The albino rats of either sex were used for these studies.

Results: The results of hepatoprotective study revealed that the SC extract showed a liver protective action against isoniazid induced toxicity.

Conclusion: The present study suggests that the extract of S. commersonnii fish has therapeutic potential in the management of drug induced liver toxicity.

KEYWORDS: Hepatoprotective, isoniazid induced toxicity, SC, Stolephorus commersonnii

Introduction

Marine environment is considered to be more biologically diverse than terrestrial environments. Approximately, 79% of the earth’s surface is covered with water. Marine environments may contain over 80% of the world’s plant and animal species. Many different marine organisms have been explored for bioactive compounds. Some vertebrate animals include fish, sharks, and snakes. Some examples of invertebrates are sponges, coelenterates, tunicates, echinoderms, corals, algae, and bryozoans. Some microorganisms include bacteria, fungi, and cyanobacteria. In recent years, the dietary fish compounds occurring in fish meat, has received much attention. There has been an increased research interest to develop hepatoprotective, cardioprotective, anticancer; and other pharmacological therapies from natural sources such as animals and plants of marine origin. Adequacy in nutrient intake in terms of quantity and quality are major determinants of health of a nation. India is undergoing nutrition
transition and is facing the dual burden of malnutrition, i.e. problem of under-nutrition and micronutrient deficiencies. Fish is the one of the food that can overcome this problem.

In the 2004, ziconotide isolated from a marine cone snail in USA, is the first food and drug administration approved drug as analgesic, which has paved the way for exploring marine-derived compounds. Drugs such as cytarabine and eribulin from a marine sponge for cancer therapy, vidarabine from sponge as antiviral drug, and lovaza from fish for hypertriglyceridemia treatment. The greatest marine tropical biodiversity is reported to be in Indo-Pacific Ocean. Important medicinal values of many marine species have been reported but a large number of them remain unexplored as yet.

It is clear that causes of many diseases are due to the “oxidative stress” that results from an imbalance between formation and neutralization of pro-oxidants. It has been well-recognized that many biochemical reactions involve the generation of reactive oxygen species in our body.

Liver diseases are one of the important worldwide health problems. Liver is a unique organ in the body and is central to the regulated metabolism as it performs detoxification of endogenous and exogenous compounds. The liver is the principal organ that is capable of converting drugs into another forms that can be readily eliminated from the body.

Certain medicinal agents, when taken in overdoses and sometimes even when introduced within therapeutic ranges, may injure the organ. Other chemical agents such as those used in laboratories and industries, natural chemical agents (e.g., microcystins), and herbal remedies can also induce hepatotoxicity. Chemicals that cause liver injury are called hepatotoxins. Main hepatotoxic drugs are paracetamol, isoniazid, carbon tetra chloride etc.

*Stolephorus commersonnii* (Commerson’s anchovy) is a schooling fish found abundant in Kerala coastal waters, apparently entering brackish water belonging to the *Stolephorus* genus of fish in the *Engraulidae* family. Whitebait or the whitebait anchovy is the common name applied to the fishes of the genera *Stolephorus*. There are approximately 96 species in the *Stolephorus* genus.

This fish is reported to contain higher content of essential amino acids, monounsaturated fatty acids, polyunsaturated fatty acids, and minerals. The medicinal values of this species have not been reported and remain unexplored. Hence, there is a necessity to explore their uses and to conduct the pharmacological studies to ascertain their therapeutic properties. Based on literature search, no study has been carried out to scientifically validate the fish *S. commersonnii* possesses hepatoprotective property. Hence this study was carried out to investigate the hepatoprotective effect of the *S. commersonnii*.

### Materials and Methods

#### Collection and Authentication

The fish was purchased from the fish market, Ernakulam, in the month of March 2015. The fish were acclimatized for 10 days and maintained in uniform climatic conditions. Animals were divided into different groups and were kept for acclimatization. They were maintained in uniform climatic conditions.

#### In vivo Hepatoprotective Activity

The albino rats were divided into five groups, each group had six animals. Group I (control) animals were administered a single daily dose of carboxymethyl cellulose. Group II received isoniazid (100 mg/kg body weight, i.p) alone whereas Group III received silymarin (50 mg/kg), the known hepatoprotective compound, along with isoniazid. Group IV received orally 300 mg/kg body weight of the fish extract along with isoniazid. Rats were treated as per the treatment protocol for a period of 21 days. The blood was collected by cardiac puncture. Rats were sacrificed for histopathological studies. Blood samples were centrifuged for 10 min at 3000 rpm to separate the serum. Serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), alkaline phosphatase (ALP), total protein, bilirubin levels estimated from the serum by using standard kits.

### Results

The percentage yield obtained after the extraction of *S. commersonnii* fish was found to be 3% w/w. The prepared extracts were subjected to preliminary analysis of chemical constituents. The fish extract contains lipids, proteins, amino acids, and cholesterol.

Rats treated with isoniazid developed a significant hepatic damage observed as elevated serum levels of hepatospecific enzymes such as SGPT, SGOT, ALP, and bilirubin and decreased level of total protein when compared to normal control. Pretreatment with silymarin, fish extract had showed good protection against isoniazid induced toxicity to liver. Test indicates a significant reduction in elevated serum levels when compared to toxic control which is evident in the Table 1.

### Extraction and Preliminary Analysis of Chemical Constituents

Fish is washed and cleaned properly. The tissue is homogenized with chloroform: methanol (2:1) to a final dilution 10 times the volume of the tissue sample (1 g in 10 ml of solvent mixture). After dispersion, the mixture is agitated for 1 h in an orbital shaker at room temperature. Then the homogenate is filtered through a Whatman No: 1 filterpaper on a Buchner funnel with a slight suction (vacuum filtration). Washed the crude extract with 0.2 of its volume of water. The filtrate is collected, transferred to a separating funnel, and allowed the solution to separate into two phases. The lower fraction is collected and evaporated under vacuum in a rotary evaporator. The extract is transferred to a dark glass container and stored in a freezer.

The preliminary analysis of fish extract was carried out for lipids, proteins, steroids and amino acids by simple qualitative methods.

#### Animals

Healthy Wistar Albino rats of either sex weighing 150–200 g were collected from the animal house of Department of Pharmaceutical Sciences (DPS), RIMSR, Puthuppally (CPCSEA NO: 1702/po/c/13/CPCSEA). Animals were fed with standard diet and water *ad libutum*. The protocol was approved at the Institutional Animal Ethical Committee (IAEC) of DPS, RIMSR, Puthuppally, with IAEC No: DPS/01/2015. The animals were divided into different groups and were kept for acclimatization. They were maintained in uniform climatic conditions.

The fish was purchased from the fish market, Ernakulam, in the month of March 2015. The fish were kept on ice until processed in the lab. The fish material was identified and authenticated by Dr. Satyen Kumar Panda, Senior Scientist, Quality Assurance and Management Division, Central Institute of Fisheries Technology, Cochin, Kerala, India.

Table
Discussion

The objective of this project is to establish scientific evidence for protective role of SC extract against the hepatotoxicity.

The hepatoprotective activity was studied by using isoniazid as hepatotoxicity inducing agent. Isoniazid is widely used as anti-tuberculosis drug, which causes hepatotoxicity even at its therapeutic doses. The mechanism of hepatotoxicity is first causes the necrosis of centrilobular hepatocytes and followed by lipid peroxidative degradation of glutathione and produce cell necrosis in liver due to the formation of toxic species by isoniazid.

Aspartate aminotransferase (AST) predominantly found in mitochondria of hepatocytes. Alanine aminotransferase (ALT) is more specific to liver, and thus is a better parameter for detecting liver injury. Serum ALP and bilirubin is also associated with liver cell damage. The ALT, AST, and ALP activity and serum bilirubin level are largely used as most common biochemical markers to evaluate liver injury. Administration of isoniazid caused a significant elevation of enzymes level such as AST, ALT, ALP, and bilirubin and has been attributed to damage the structural integrity of liver, because they are cytoplasmic in location and released into circulation after cellular damages, indicating the development of hepatotoxicity. Histopathological profile of liver from vehicle treated rat [Figure 1] showed liver tissue with portal triad sinusoids and normal hepatocytes which is the normal appearance whereas isonicotinic acid hydrazide intoxicated rats [Figure 2] showed foci of necrosis of hepatocytes and infiltration of lymphocytes.

The fish extract was administered orally 300 mg/kg for Wistar rats daily. Silymarin (50 mg/kg) was selected as the standard drug which is well-known hepatoprotective drug. The hepatoprotective activity was confirmed by the significant reduction in the elevated levels of diagnostic biochemical markers like SGPT, SGOT, ALP, and bilirubin [Graphs 1–4] and increase in the total protein level [Graph 5]. In addition, the histopathological observations have shown that the section of liver in silymarin treated group [Figure 3] showed no necrotic changes except for occasional focal mild necrotic areas and no inflammatory cell infiltrate and in test drug treated groups [Figure 4] shows intact architecture, only occasional minor necrotic foci; otherwise liver tissue appears normal, which is similar to silymarin treated group. Hence, the present study suggests that the extract of S. commersonnii fish has therapeutic potential in the management of drug induced liver toxicity. As this drug is unexplored further studies on its active constituents, its isolation, purification, and characterization along with investigations is needed to provide some additional

Table 1:

| Groups          | SGOT   | SGPT   | ALP       | Bilirubin | Total protein |
|-----------------|--------|--------|-----------|-----------|--------------|
| Normal control  | 27.14±0.529 | 31.86±0.518 | 97.913±0.670 | 0.569±0.043 | 6.848±0.052  |
| Toxic control   | 96.41±0.716 | 112.13±0.616 | 126.418±0.487 | 1.379±0.056 | 3.676±0.061  |
| Standard        | 38.92±0.514** | 53.48±0.416** | 93.083±0.593** | 0.900±0.046** | 6.865±0.057** |
| Test            | 48.41±0.592** | 76.44±0.466** | 114.315±0.547** | 1.067±0.037** | 6.783±0.042** |

Values are mean±SEM (n=6) one-way ANOVA followed by Tukey test. **Moderately significant at P<0.01, ***Highly significant at P<0.001. All values are compared with toxicant. SEM=Standard error of mean, SGOT=Serum glutamic oxaloacetic transaminase, SGPT=Serum glutamic pyruvic transaminase, ALP=Alkaline phosphatase
insight into the mechanism of action by which it exhibit these activities.

In addition, this study will be beneficial for the mankind, as this fish has a therapeutic potential for curing the drug induced liver toxicity. Most of the people are consuming drugs daily, so liver injury can be occurred. If this fish is included on their daily food routine, it will be good for their liver, we can prevent the toxicity. Not only medicine but also change in the food habit can prevent a disease.

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Conflicts of Interest
There are no conflicts of interest.

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