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Lipid Profile of Liver Oil of the Sickle-Finned Chimaera (*Neoharriotta pinnata*) of the Arabian Sea

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ABSTRACT

Liver oil from the deep sea, sickle-finned chimaera, *Neoharriotta pinnata* was analyzed to describe its lipid composition. Triacylglycerols, di- and monoacyl glyceryl ethers, tocopherol and squalene were the major lipid components observed. Only trace levels of polar lipids were detected. Saturated, mono- and poly-unsaturated fatty acids constituted 19, 57 and 22% of the total fatty acids, respectively. The high squalene (52% of oil) and tocopherol (2.5% of oil) contents suggest that oil from the holocephalian would find great applications in the nutraceutical and pharmaceutical industries.

Keywords: alkylglycerols, fatty acids, fish, squalene, tocopherol

INTRODUCTION

Chimaeras are an important resource among the marine fishes caught in India. While the marine waters up to 50 m depth have been extensively studied, the waters beyond this depth remain unexplored. Statistics (Fowler *et al.* 2005) have shown that over 30,000 tonnes of chimaeras, pelagic sharks and certain species like squaline sharks (inhabiting waters beyond 600 m) available in India's Exclusive Economic Zone (EEZ) have hardly been exploited. While shark fishing gained momentum over the years, much of their commercial value has been limited to the sale and supply of shark fins. The nutraceutical values associated with the liver oils of sharks and chimaeras, from the Indian EEZ remain unexplored.

Therapeutic use of shark liver oil is evident from its use for centuries as a remedy to heal wounds and fight flu (Neil *et al*. 2006). Japanese seamen called it *samedawa*, or "cure all". Shark liver oil is being promoted worldwide as a dietary supplement to boost the immune system, fight off infections, to treat cancer and to lessen the side effects of conventional cancer treatment (Szostak and Szostak-Weqierek 2006). These days, more emphasis is laid on the nutritive benefits of shark liver oils especially on the omega 3 polyunsaturated fatty acids (PUFAs) (Anandan *et al.* 2007) and alkylglycerols (AKGs) (Pugliese *et al*. 1998) contained in them due to the high rise of inflammatory disorders like arthritis, asthma and neurodegenerative diseases like Alzheimer's, Parkinson's and schizophrenia. Higher concentrations of AKGs in shark liver oils are now considered to be responsible for its high immune boosting ability (Pugliese *et al*. 1998). These AKGS are essentially a class of lipids with an ether linkage and a glycerol backbone. In addition, shark liver oils also contain antioxidant vitamins and squalamine (Brunel *et al*. 2005), a substance which has shown a promising behavior towards fighting breast, lung, brain, and skin cancers (specifically melanoma) by choking off the tumor's blood supply. The pharmaceutical values associated with shark liver oils are abundant; they form the active ingredients of many different formulations ranging from vitamin supplements to skin-based ointments and creams (Neil *et al*. 2006).

The aim of the present study was to determine the lipid composition of the deep sea chimaera, *Neoharriotta pinnata* (Family: Rhinochimaeridae; Order: Chimaeriformes; Class: Holocephali), with special reference to the hydrocarbon squalene, the AKGs, tocopherol and PUFA, caught from the Arabian Sea. Lipid characterization would provide insight into the abundance of specific lipids in the liver of these deep sea holocephalians. These results could assist industry with the exploitation of the liver of these chimaeras, currently regarded by some as waste.

MATERIALS AND METHODS

N. pinnata was caught from the Arabian Sea, off the Alleppey coast, from 624 m depth during Cruise 250 on the FORV Sagar Sampada, in January 2006. An Expo model trawl net was used to catch the sickle-finned chimaera and the liver tissue excised immediately was frozen at -20°C onboard the vessel. The liver was then brought to the laboratory for further analyses. All chemicals and reagents used were obtained from Merck (Darmstadt, Germany). The chemical standards used for the analyses were purchased from Sigma-Aldrich Chemical Inc. (St. Louis, MO, USA).

Oil extraction

Accurately weighed liver of *N. pinnata* was subjected to lipid extraction by the method of Folch *et al.* (1957). Briefly, minced liver was homogenized in a 2:1 (v/v) mixture of chloroform-methanol and filtered. 20% water was added to this mixture and the layers were allowed to separate overnight. The aqueous layer was discarded the following day and the total solvent extract was concentrated (i.e. solvents were removed *in vacuo*) using rotary evaporation at 40°C. The oil was made up to a known volume in chloroform and stored in amber-coloured bottles under nitrogen at -20°C. A portion of the oil was saponified (Hallgren and Larsson 1962), in a mixture of 150% potassium hydroxide (w/v) and absolute ethanol for 2 h in a water bath at 75°C under an inert atmosphere of nitrogen. The resulting mixture was extracted with ether, water-washed, dried over anhydrous sodium sulphate and finally condensed to a known volume. A small portion of the ether layer was air dried to estimate the fraction of the non-saponifiable matter (NSM) present in the oils.

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Short Communication

Analysis of lipid components

Aliquots of the ether extract or the diluted crude liver oil were analyzed using an Iatroscan MK-6s (M/s. Mitsubishi Kagaku Iatron Inc. Tokyo, Japan) to determine the abundances of individual lipid classes (hydrocarbons, alkoxyglycerols, triacylglycerols, fatty acids) (Bakes and Nichols 1995). Samples were applied in triplicates to silica gel SIII chromarods (5 µm particle size) using 1 µl disposable micropipettes. Chromarods were developed in a glass tank lined with pre-extracted filter paper. The solvent system used for the lipid separation was chloroform-methanol-water-ammonia (47:23:4:0.25 v/v/v/v), a mobile phase separating polar lipids. A second non-polar solvent system of hexane-diethyl ether (60:15 v/v) was also used to resolve the non-polar lipid components. After development, the chromarods were oven dried and analyzed immediately to minimize adsorption of atmospheric contaminants. The flame ionization detector (FID) was calibrated for each compound class (squalene, monopalmitoyl-rac-glycerol, oleic acid, tripalmitin, α-tocopherol, phosphatidylcholine). The peaks obtained via a Chromatocorder were quantified and tabulated.

Analysis of fatty acid profile

Aliquots of the ether extract of the liver oil (three replicates) were methylated using BF_3-methanol and the resulting fatty acid methyl esters (FAME) (Davidson and Cliff 2002) were injected into the Trace GC Ultra gas chromatograph (M/s. Thermo Electron Co., Milan, Italy) equipped with Perkin Elmer Elite 225, 50% cyanopropyl phenyl – 50% methyl capillary column (30 m × 0.25 mm i.d.), a flame ionization detector (FID) and a split/splitless injector. Nitrogen was the carrier gas. Briefly, the aliquots were injected in splitless mode at an oven temperature of 110°C. After 4 min the oven temperature was raised to 240°C at 2.7°C/min. Peaks were analyzed and quantified using Chromcard software, with the help of running authentic standards.

Statistical analysis

Data obtained from three replicates of the liver oil sample were subjected to descriptive statistics using SPSS 16.0 Software Package and the values were expressed as mean ± SD.

RESULTS

The lipid composition of the oil extracted from the liver of the sickle-finned chimaera *N. pinnata*, from the Arabian Sea was analyzed (**Table 1**). The oil contained a high fraction of non-saponifiable matter (NSM), nearly 78% of the total lipid. Hydrocarbons (HCs), fat soluble vitamins and alkylglycerols (AKGs) were the major components of the NSM. The hydrocarbon fracton of NSM was predominated by the isoprenoid squalene – 52% of the total lipid. An important finding was the presence of high amounts of the antioxidant vitamin α– tocopherol (nearly 3% of the total lipid) in the liver oils of the chimaera. Vitamin A was present at less than 1%. AKGs comprising of triacylglycerols and both di- and monoacyl glyceryl ethers were present at 4, 3 and 10% of the total lipid, respectively. Cholesterol was the major sterol observed at 5% of the total lipid levels. Polar lipids and free fatty acids were low in abundance (<2%).

Table 1 Lipid composition of liver oil of *Neoharriotta pinnata*.

Crude fat content (w/w)	69.28 ± 3.33
Lipid composition (as % total lipid)	
Non-saponifiable matter	78.01 ± 3.43
Hydrocarbon (squalene)	52.43 ± 8.29
Triacylglycerols	3.89 ± 3.07
Diacylglyceryl ether	3.46 ± 4.23
Monoacylglyceryl ether	10.61 ± 3.80
Sterol (cholesterol)	5.73 ± 2.15
Vitamin E (tocopherol)	2.55 ± 0.59
Vitamin A (retinol)	0.96 ± 0.88
Saponifiable matter	22.86 ± 0.45
Free fatty acids	0.85 ± 0.20
Total fatty acids	21.18 ± 4.85
Polar lipids	0.83 ± 0.06

Results are mean % of three replicates of the liver oil ± S.D.

Table 2 Total fatty acid composition of liver oils of *Neoharriotta pinnata* collected from the Arabian Sea. Results are mean % of 3 replicates of the liver oil ± S.D.

Fatty acids	% total fatty acids
14:0	0.72 ± 0.11
14:1	0.30 ± 0.14
15:0	0.98 ± 0.12
15:1	0.10 ± 0.10
16:0	12.36 ± 0.40
16:1	4.71 ± 0.46
17:0	0.30 ± 0.21
17:1	3.10 ± 0.10
18:0	3.60 ± 0.63
18:1(n-9)	29.68 ± 0.72
18:2(n-6)	2.46 ± 0.67
20:0	0.40 ± 0.40
20:1	11.35 ± 0.68
20:3(n-3)	5.20 ± 0.64
20:5(n-3)	4.53 ± 0.44
22:0	0.67 ± 0.20
22:1	6.22 ± 0.70
22:6(n-3)	10.03 ± 0.71
24:1	2.40 ± 0.52
Others	0.88 ± 0.62
Total	100.00
Sum saturates	19.03
Sum monounsaturates	57.87
Sum polyunsaturates	22.22
Total fatty acid (mg/g)	211.80

The fatty acid and total fatty acid content of the liver oil of *N. pinnata* was analysed (**Table 2**). High levels of monounsaturated fatty acids were observed (57% of the total fatty acid) with 16:1, 18:1(*n*-9), 20:1 and 22:1 being the dominant fatty acids. Minor levels of 17:1 and 24:1 were also detected. The poly-unsaturated fatty acids (PUFA) constituted 22% of the total fatty acids with 20:3(*n*-3), 20:5(*n*-3) and 22:6(*n*-3) being the major contributors. Eicosapentaenoic acid (EPA, 20:5(*n*-3)) and docosahexaenoic acid (DHA, 22:6(n-3)) were the most abundant of the PUFAs, comprising up to 15% of the total fatty acids in *N. pinnata*. Saturated fatty acids constituted 19% of the total fatty acids with 16:0 and 18:0 being the major contributors.

DISCUSSION

The composition of liver oils from the sickle-finned chimaera *N. pinnata* from the Arabian Sea has not been previously documented. These chimaeras are frequently caught in deep-sea fishery trawls along the southwest coast of India. The liver oil from *N. pinnata* was found to contain high levels of the HC squalene which is typical of liver oils from deep-water elasmobranchs inhabiting water depths between 600 and 1000 m (Bakes and Nichols 1995). Triacylglycerols, di- and monoacyl glyceryl ethers together formed 18% of the liver oils of *N. pinnata*. According to Deprez *et al.* (1990), the levels of these specific lipids have been found to vary from 18% in certain species of dogfish sharks (*Centrophorus scalpratus*) to as high as 90% in Plunket (*Somniosus pacificus*) and sleeper sharks (*Centroscymnus plunketi*). The role of specific lipids and hydrocarbons as buoyancy regulators in the liver of deep sea sharks has been documented (Malins and Barone 1970; Phleger and Grigor 1990) and it is apparent that different sharks regulate liver lipid composition to maintain buoyancy. The levels of squalene, triacylglycerols, di- and monoacyl glyceryl ethers and the unusually high amounts of tocopherol in the liver oils of chimaera could also be affected by the dietary intake of specific components and seasonal variations (Kayama *et al*. 1971; Hayashi and Takagi 1981). Phleger and Grigor (1990) showed

that *Hoplostethus atlanticus* found at similar depths to these deep-sea chimaeras use lipid deposits to control buoyancy.

The total fatty acid content obtained from the liver oil of *N. pinnata* was 211 mg/g. Previous reports by Buranudeen and Richards-Rajadurai (1986) confirmed the variations in the total fatty acid content in certain *Centrophorus* species (possessing high squalene contents in their liver oils), to range from 95–600 mg/g. The fatty acids in the liver oils of *N. pinnata* were mainly the mono- and poly-unsaturated types. The role of EPA and DHA in lipid fluidity has been previously documented (Russell 1990) and the high levels of DHA in chimaeras and sharks may complement the levels of AKGs in them and play a role in maintaining their fluidity. AKGs are important in the treatment of haematopoiesis and radiation sickness (Devaraj and Jialal 2000; Pedrono *et al*. 2004).

It has also been proved that long chain *n*-3 PUFAs (James *et al*. 2003) lower the incidence of inflammatory diseases such as asthma and arthritis (Calder 2006). These dietary fatty acids are known to reduce the levels of arachidonic acid metabolites and lower the formation of proinflammatory compounds, like prostaglandins and leukotrienes, by blocking their activity (Olivera *et al.* 2004). Early studies reviewed by Stamp *et al.* (2005) and Calder (2006) attributed the anti-inflammatory effects of fish oils to competition with arachidonic acid for production of inflammatory eicosanoids. EPA and DHA contained in fish oils also help to increase levels of digestive enzymes in the body thereby providing nutrients needed to build anti-inflammatory prostaglandin series 1 and 3, which helps in weight loss (Simopoulos 1991).

In addition, the high levels of squalene (Ko *et al*. 2002) and tocopherol (Devaraj and Jialal 2000) in the liver lipids of *N. pinnata* help reduce inflammation by decreasing C-reactive protein levels by blocking the activity of TNF-α (tumour necrosis factor-alpha) series 2-prostaglandins (PGE-2) and cyclooxygenases (James *et al*. 2003). The role of squalene as an antilipidemic agent (Qureshi *et al*. 1996) and membrane stabilizer has been well documented (Sabeena *et al*. 2004).

CONCLUSION

Studies on the pharmacological properties of liver oils from sharks, inhabiting the waters beyond 600 m depth, of the Arabian Sea are scanty. The liver oils of *N. pinnata* analyzed contained high fractions of health boosting non-saponifiable matter (78% of total lipid) predominated by the hydrocarbon squalene (52% of total lipid) that could render beneficial effects. Further investigations on lipid composition of liver oils of *N. pinnata* from different geographical locations at different times of year are necessary for better characterization of liver oils from these deep sea chimaeras. The abundance of these lipids from diverse sources, including elasmobranchs, many marine oils and various mammalian species, may provide future stimulation for the pharmaceutical, health product and related industries to prepare a wide range of products for the betterment of the human race.

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