ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES OF LIVER OILS OF FOUR SHARK SPECIES FROM INDIAN EEZ

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ABSTRACT

The analgesic and anti-inflammatory properties of liver oils of four different sharks, namely Neohariotta raleighana, Centrosymnus crepidater, Apristurus indicus *and* Centrophorus scalpratus, *captured from the Arabian Sea and the Indian Ocean were evaluated. While the analgesic property was determined using the acetic acid-induced mouse writhings and hot-plate reaction time, the anti-inflammatory activity was evaluated using the formalin-induced rat-paw edema. The oils examined were found to possess significant (*$P < 0.05$*) analgesic activity against acetic acid-induced writhings and hot-plate reaction in mice. In the formalin-induced edema, a significant (*$P < 0.05$*) inhibition of inflammation was observed between the 2nd and 4th hour showing 58–65% inhibition. These results suggest that liver oils of sharks from Indian waters are effective as analgesic and anti-inflammatory agents. The role of lipid components (squalene, alkylglycerols and polyunsaturated fatty acids) on anti-inflammatory and antinociceptive properties is highlighted. Inhibition of the synthesis of prostaglandins and other inflammatory mediators which probably account for the properties is discussed.*

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PRACTICAL APPLICATIONS

Studies on the pharmacological properties of liver oils from sharks, inhabiting the waters beyond 600 m depth of the Indian Exclusive Economic Zone are scanty. Shark liver oils contain high fractions of health-boosting unsaponifiable matter and unsaturated fatty acids that could render beneficial effects. Results suggest that these oils possess excellent anti-inflammatory and peripheral antinociceptive effects that may contribute to its use in the treatment of arthritis and other inflammatory disorders.

INTRODUCTION

Oceans are unique resources that provide a diverse array of natural products (Marris 2006), primarily from invertebrates such as sponges, tunicates, bryozoans and mollusks, as well as from marine bacteria and cyanobacteria. While bioactive compounds of varied origin have been explored from deep sea resources worldwide, the discovery of natural drugs from the fishery resources of the Indian Exclusive Economic Zone (EEZ) is still in its infancy. Over the past few years, the perception of marine nutraceuticals (Shahidi 2007) to the health care professional and consumer has been popularized to fish oils from the greatest predators of the sea – the sharks.

Sharks are an important resource among the marine fish species caught in India. While the marine waters up to 50 m depth have been studied extensively, the waters beyond this depth remain unexplored. Statistics (Fowler *et al.* 2005) have shown that over 30,000 tons of pelagic shark and certain species like squalene sharks (inhabiting waters beyond 600 m) available in India's 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 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 infections, to treat cancer and to lessen the side effects of conventional cancer treatment. 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 because of the high rise of inflammatory disorders such as arthritis, asthma and neurodegenerative diseases like Alzheimer's, Parkinson's and schizophrenia. Even though reports have confirmed the role of shark liver oils in lowering the

incidence of these diseases, the exact mechanisms involved in these disorders are yet to be explored.

Higher concentrations of AKGs in shark liver oils are now considered to be responsible for their 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 toward fighting cancers of the breast, lung, brain and skin (melanoma specifically) 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 evaluate the analgesic and anti-inflammatory effects of four different liver oils of sharks belonging to the Indian EEZ and to identify the components of the oil that is responsible for these activities. The analgesic and anti-inflammatory activities of liver oils from *Neoharriotta raleighana* (NR), *Centrosymnus crepidater* (CC), *Apristurus indicus* (AI) and *Centrophorus sculpratus* (CS) sharks caught from the Arabian Sea and the Indian Ocean were compared. As the information available with regard to these properties is relatively scanty, an attempt has been made to explore their ability as therapeutic agents.

MATERIALS AND METHODS

Materials

The shark species were caught beyond 600 m depth during Cruises 250 and 252 on the Fishery and Oceanographic Research Vessel Sagar Sampada from the southwest and eastern coasts of India. Expo model trawl nets were used to catch these deep sea fish species and they were immediately frozen at –20C onboard the vessel, and brought to the laboratory for further analyses. The catch details of the four different shark species, whose liver oils were analyzed for their analgesic and anti-inflammatory potentials, are shown in Table 1. All chemicals used were obtained from Merck (Darmstadt, Germany). Paracetamol and ibuprofen, the standard reference drugs used for the animal experiments, were purchased from Sigma-Aldrich Chemical Inc. (St. Louis, MO).

Methods

Oil Extraction. The livers of each of these sharks were excised and weighed separately. Lipid extraction was achieved by following the method of

TABLE 1.
DETAILS OF SHARK SPECIES COLLECTED SHOWING THEIR COMMON NAMES, LOCATION (LATITUDES AND LONGITUDES) AND DEPTH

S.No.	Scientific name (abbrev.)	Common name	Region	Lat (ºN)	Long (ºE)	Depth (m)
1	*Apristurus indicus* (AI)	Small-belly catfish	Azhikkal	12°04′	74°16′	735
2	*Centrophorus sculpratus* (CS)	Endeavour dogfish	Diglipur	13°21′	93°07′	695
3	*Centrosymnus crepidater* (CC)	Deep sea dogfish	Kasargode	12°25′	74°07′	740
4	*Neoharriotta raleighana* (NR)	Long-nosed ratfish	Alleppey	09°17′	75°38′	624

Folch *et al.* (1957). Briefly, minced liver was homogenized in a 2:1 (v/v) mixture of chloroform-methanol and filtered. Then, 20% water was added to this mixture and the layers were allowed to separate. The aqueous layer was discarded and the solvent was completely evaporated to obtain the oil. The oils were stored in amber-colored bottles under nitrogen at −60C. 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 75C under nitrogen. The resulting mixture was extracted with ether, water-washed, dried over anhydrous sodium sulfate and finally condensed to a known volume. A small portion of the ether layer was air-dried to estimate the fraction of the unsaponifiable matter (USM) present in the oils.

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 (HCs, alkoxyglycerols, triacylglycerols, fatty acids [FAs]; Bakes and Nichols 1995). Samples were applied in triplicate 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. A nonpolar solvent system of hexane-diethyl ether (60:15, v/v) was used to resolve the 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 class of compounds (squalene, monopalmitoyl-rac-glycerol, oleic acid, tripalmitin). The peaks obtained via Chromatocorder were quantified and tabulated.

FA Methyl Esters. Aliquots of the ether extract were methylated using BF_3-methanol and the resulting FA methyl esters (Bakes and Nichols 1995) were subjected to gas chromatography (M/s. Thermo Electron Corporation, Milan, Italy) equipped with Perkin Elmer Elite 225 (Perkin Elmer Life and Analytical Services, Watham, MA) 50% cyanopropyl phenyl – 50% methyl

capillary column (30 m × 0.25 mm inner diameter) FID and a split/splitless injector. Nitrogen was the carrier gas. Samples were injected in splitless mode at an oven temperature of 110C. After 4 min, the oven temperature was raised to 240C at 2.7C/min. Peaks were quantified with Chromcard software by comparing retention time data with those obtained for authentic standards.

Experimental Animals. Wistar strain male albino rats (120–200 g) and mice (35–40 g) were used in the experiments. They were housed individually in polypropylene cages under hygienic conditions and were provided food and water *ad libitum*. The animals were maintained on a 12:12-h light : dark photoperiod under standard conditions of temperature and ventilation. The experiments were performed as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, New Delhi, India, and with the approval of the Institutional Animal Ethics Committee.

Experimental Protocol. The analgesic and anti-inflammatory activities of the shark liver oils were assessed after checking their lethal dosages in rats and mice as animal models. Analgesic activity was determined using the acetic acid-induced writhing and hot-plate tests, while the anti-inflammatory activity was determined using the formalin-induced rat-paw edema test.

Toxicity Studies. Acute toxicity of the four shark liver oils was carried out on male albino rats and mice using Karber's arithmetical method for the determination of LD_{50} (lethal dose that causes mortality by 50%) (Turner 1965a). In this assay, increasing doses of the test substance, oil along with the vehicle dimethylsulfoxide (DMSO) (oil : DMSO, 4:1), were administered orally to groups of four animals at different doses (1.0–7.0 g/kg). The animals were observed for 1 week, the number of survivors was counted and the optimum average dosage was determined.

Acetic Acid-Induced Writhing Assay. In vivo determination of antinociceptive activity was carried out using the abdominal constriction test (Koster *et al.* 1959). The mice were divided into six groups of five animals each after initial screening. All shark liver oils were administered orally (50 mg/0.2 mL/animal) as a suspension in DMSO (oil : DMSO, 4:1) 1 h prior to the intraperitoneal administration of acetic acid (0.6% v/v). Ten minutes after the administration, the number of constrictions per animal was recorded at 20 min. Control animals received an equal volume of vehicle. The standard reference drug paracetamol was administered at 100 mg/kg body weight. Antinociceptive activity was reported as percent inhibition of constrictions compared with the placebo control group.

Hot-Plate Activity. The analgesic activity was investigated in male albino mice using the hot-plate test (Turner 1965b). The mice were divided into six groups of five animals each after initial screening. Each of the shark liver oils was administered orally at a dose of 50 mg/0.2 mL/animal as a suspension in DMSO (oil : DMSO, 4:1). Control animals received an equal volume of the vehicle. One group received the reference drug paracetamol at 100 mg/kg body weight. The animals were dropped gently on the hot-plate maintained at 53 ± 1C; this was done 5 min prior to the administration of the vehicle, oils and paracetamol, and at 30, 60, 90, 120, 150 min following administration. The time between placement and the first sign of paw licking or jumping was recorded as latency. The basal latencies were 6–10 s. A cut-off time of 30 min was established to prevent injury to the paws. The mean values were recorded.

Anti-Inflammatory Activity. The anti-inflammatory activity was determined, by the method of Hunskaar and Hole (1987), using the formalin-induced rat-paw edema test. Male albino rats weighing between 160–180 g were divided into five groups of six animals each. The oil along with the vehicle DMSO (oil : DMSO, 4:1) was administered orally at a dose of 1.5 g/kg body weight prior to the induction of inflammation by the subcutaneous injection of 0.1 mL sterile saline solution of 3.5% formalin in the right hind paw. The control group received sterile saline solution (1 mL 0.9% NaCl solution), while the reference drug ibuprofen at a dose of 100 mg/kg body weight was administered intraperitoneally to the standard group at least 30 min before the induction of edema. Paw sizes were measured with a calibrated screw gauge before the administration of formalin, then thereafter at 1, 2, 3 and 4 h after the injection of the inflammatory agent. The average size of the paw measured in millimeters was calculated from three measurements which did not differ by more than 1%. These individual measurements allowed us to determine the average paw size for each group (s_m) and then the percentage of edema by comparison with the average size obtained for each group before any treatment (s_o).

Percentages of inflammation-inhibition were obtained for each group using the following calculation:

$$\frac{[(s_m - s_o)\text{control} - (s_m - s_o)\text{treated}]}{(s_m - s_o)\text{control}} \times 100 \qquad (1)$$

where s_m is the mean paw size for each group after formalin treatment and s_o is the mean paw size obtained for each group before the treatment (Owolabi and Omogbai 2007).

Statistical Analyses. All data were expressed as mean ± SD and analyzed statistically by one-way analysis of variance using Duncan's test with a level of significance set at $P < 0.05$. The statistical software, SPSS for Windows version 16 (SPSS Inc., Chicago, IL), was employed for the analyses. Pearson's correlation test was performed between the various lipid components analyzed and the inflammation-inhibitory values to determine the factor(s) responsible for the observed pharmaceutical effects. A positive correlation was established when the level of significance was set at $P < 0.05$ or $P < 0.01$ as the case may be.

RESULTS

Acute Toxicity of the Oils

In the acute toxicity trial, no mortality was observed for doses (oral administrations) of up to 3.8 g/kg body weight for either mice or rats. No significant changes in the body weight were observed at this dose. However, 50% mortality was observed for both rats and mice at a dose of 6.2 g/kg body weight after 1 week. Based on these observations, the oils were administered at an optimum average dose of 1.5 g/kg body weight.

Lipid Composition of Oils

The lipid composition of oils extracted from the liver of four species of deep sea sharks found in southern Indian waters was determined. The oils of NR, CS and CC recorded high USM content of 78, 73 and 60%, respectively (Table 2). However, AI oil had the lowest content of USM (25%) among

TABLE 2.
LIPID COMPOSITION OF SHARK LIVER OILS (VALUES ARE MEAN ± SD)

Species (abbrev.)	Lipid (% tissue)	NSM (% lipid)	HC (% lipid)	AKG (% lipid)	FA (% lipid)
Apristurus indicus (AI)	70.58 ± 1.87	25.29 ± 5.71	20.11 ± 2.31	12.20 ± 5.51	68.83 ± 7.22
Centrophorus sculpratus (CS)	79.46 ± 2.27	73.62 ± 9.52	67.42 ± 5.84	18.52 ± 7.31	24.20 ± 6.67
Centrosymnus crepidater (CC)	77.43 ± 2.85	60.71 ± 8.69	52.76 ± 7.34	20.45 ± 6.77	28.21 ± 5.24
Neoharriotta raleighana (NR)	69.28 ± 1.33	78.01 ± 5.43	62.43 ± 5.29	21.13 ± 5.24	22.01 ± 4.85

NSM, nonsaponifiable matter; HC, hydrocarbons; AKG, alkylglycerols; FA, fatty acid, SD, standard deviation.

the four oils examined. AKGs and hydrocarbons (HCs), predominantly the isoprenoid squalene, were the major components of the USM. HC content varied significantly among the species analyzed. Oils of AI species recorded the lowest amount of squalene at 20.1%, while that of CS was 67.4%. Oils of CC and NR species contained 52 and 62% squalene, respectively. AKGs comprising both mono- and di-alkoxyglycerols were present in all shark species at levels between 12.2 and 21.1%. Polar lipids were either present in low abundance (<2%) or were not detected in the extracted oils.

The FA and total FA content of the four species of shark are given in Table 3. The total FAs ranged from 22 to 28% of the total lipid in NR, CS and CC species, whereas it was as high as 68% in the liver oils of AI species. The predominant FAs in all species were the monounsaturates (MUFA) ranging from 55 to 67% with 18:1(n-9), 20:1 and 22:1 being the major FAs. PUFA levels varied from 10 to 22% of the total FA content in oils.

Analgesic Activity of Oils

All the four shark liver oils (AI, CC, CS and NR) exhibited a highly significant ($P < 0.05$) analgesic activity when compared with the control group. Table 4 illustrates the antinociceptive activity of the four shark liver oils, in the acetic acid-induced constrictions in mice, when administered at 1.5 g/kg body weight. The analgesic behavior of AI, CC oils and that of the standard drug paracetamol were significantly different ($P < 0.05$) from each other and from CS and NR oils. Even though there was no significant difference in activity between CS and NR oils, they differed significantly ($P < 0.05$) from AI, CC oils and paracetamol. While mice treated with AI oil showed only a 29.5% inhibition, those treated with CC, CS and NR oils showed 52.7, 59.0 and 57.8% inhibitions, respectively ($P < 0.05$). Paracetamol at 100 mg/kg body weight showed 47.2% inhibition.

The analgesic activity for all the four oils in the hot-plate test, i.e., in the latency versus time test, was similar. However, only NR and CS oils exhibited an analgesic behavior (Fig. 1) similar to that of the standard drug paracetamol (latency time of 8.3 ± 0.5 s). NR, CS and CC oils showed a significant amount of ($P < 0.05$) analgesic activity from the 60th to 90th minute. While mice treated with AI oil showed significant ($P < 0.05$) analgesic behavior up to 90 min of its administration, its effect started to decline afterwards. The latency time for control mice was 2.6 ± 0.5 s, while NR, CS, CC and AI rats showed 9.3 ± 0.0, 8.6 ± 0.5, 8.0 ± 0.0 and 6.3 ± 0.5 s, respectively, from the 60th to 90th minute of the treatment. Of the four oils analyzed, only the animals treated with AI oil showed the least antinociceptive activity.

TABLE 3.
TOTAL FATTY ACID COMPOSITION OF LIVER OILS FROM DEEP-SEA SHARKS COLLECTED FROM INDIAN WATERS*

Fatty acid	Percentage composition (as % total fatty acid)			
	Apristurus indicus	*Centrosymnus crepidater*	*Centrophorus sculpratus*	*Neohariotta raleighana*
14:0	0.65	1.17	0.21	1.72
14:1	0.82	0.16	1.30	0.30
15:0	1.00	1.00	0.06	1.98
15:1	0.70	0.11	0.54	0.10
16:0	14.15	12.43	10.82	12.36
16:1	2.89	4.00	3.34	4.71
17:0	0.15	0.35	0.16	0.30
17:1	2.93	4.98	3.82	3.10
18:0	9.79	5.65	4.17	3.60
18:1(n-9)	16.34	24.11	33.43	27.68
18:2(n-6)	1.39	ND	1.68	2.46
20:0	0.21	0.47	0.31	0.40
20:1	23.85	15.99	15.87	11.35
20:3(n-3)	7.15	5.82	8.75	5.20
20:5(n-3)	0.62	0.88	1.87	4.53
22:0	0.32	0.37	0.28	0.67
22:1	10.17	14.01	7.46	6.22
22:6(n-3)	3.88	4.08	3.10	10.03
24:1	1.50	2.42	1.83	2.40
Others	1.50	1.99	1.01	0.88
Total	100.00	100.00	100.00	100.00
Total saturates	26.26	21.43	18.01	21.03
Total monounsaturates	59.20	65.79	68.59	49.96
Total polyunsaturates	13.04	10.79	15.40	22.22
Total fatty acid (mg/g)	687.82	282.71	242.34	220.10

* All values are expressed as percentage of the total fatty acids unless otherwise stated. gas chromatography results are subject to an error of ±1%.
ND, not detected.

Anti-Inflammatory Activity of Oils

The percentage inhibition in the formalin-induced rat-paw edema is shown in Table 5. In the formalin-induced inflammation, the oils showed a peak inhibition of inflammation at the third hour. Significant ($P < 0.05$) inhibition of inflammation was shown by the standard drug ibuprofen and the four shark liver oils within 1 hour from the onset of inflammation. Ibuprofen showed a maximum inhibitory activity at the second hour (64.5%). CS and NR oils showed 65.0% reduction in edema at the third hour while AI oil was able to reduce it by only 54.2%. At the fourth hour, while all the oils as well as the

TABLE 4.
THE EFFECT OF SHARK LIVER OILS AND PARACETAMOL (STANDARD REFERENCE DRUG) ON ACETIC ACID-INDUCED WRITHING TEST IN MICE

Treatment	Number of writhes	Inhibition (%)
Control	84.6 ± 1.52[a]	–
Paracetamol	44.6 ± 3.05[b]	47.27
AI	59.6 ± 2.08[c]	29.51
CC	40.0 ± 2.00[d]	52.75
CS	34.6 ± 2.08[e]	59.05
NR	35.6 ± 3.05[e]	57.83

Inhibition is reported as percent with respect to control. Oils of *Apristurus indicus* (AI), *Centrosymnus crepidater* (CC), *Centrophorus sculpratus* (CS) and *Neohariotta raleighana* (NR) were administered orally at 1.5 g/kg and paracetamol at 100 mg/kg animal weight. Values are mean number of writhes ± standard deviation ($n = 5$ per group).

[a,b,c,d] Number of writhes with different superscripts are significantly different ($P < 0.05$).

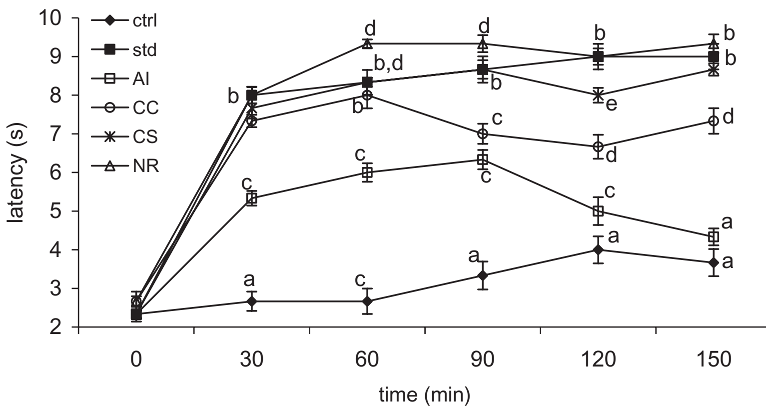

FIG. 1. EFFECT OF SHARK LIVER OILS ON THE HOT-PLATE REACTION TIME VERSUS THE BASAL LATENCY(S). OILS OF *APRISTURUS INDICUS* (AI), *CENTROSYMNUS CREPIDATER* (CC), *CENTROPHORUS SCALPRATUS* (CS) AND *NEOHARIOTTA RALEIGHANA* (NR) WERE ADMINISTERED ORALLY AT 1.5 g/kg ANIMAL; PARACETAMOL AT 100 mg/kg ANIMAL. DATA ARE EXPRESSED AS MEAN LATENCY ± SD VALUES

Latency values with different superscripts are significantly different ($P < 0.05$). Ctrl, control; std, standard; SD, standard deviation.

TABLE 5.
INHIBITORY EFFECTS OF SHARK LIVER OILS ON THE FORMALIN-INDUCED RAT-PAW EDEMA

Percentage inhibition				
Treatment	1 h	2 h	3 h	4 h
Ibuprofen	62.94 ± 0.75[a]	64.58 ± 1.09[a]	51.29 ± 3.18[a]	55.63 ± 1.86[a]
AI	20.97 ± 2.11[b]	27.95 ± 0.65[b]	54.23 ± 3.55[a]	39.42 ± 0.94[b]
CC	50.20 ± 1.49[c]	58.30 ± 1.15[c]	63.18 ± 1.40[b]	61.17 ± 0.75[c]
CS	56.18 ± 1.62[d]	60.31 ± 0.60[d]	65.72 ± 0.56[b]	69.51 ± 0.78[d]
NR	46.12 ± 0.49[e]	58.11 ± 0.43[c]	65.02 ± 5.81[b]	48.68 ± 3.89[e]

Values are mean percentage inhibition ± standard deviation ($n = 6$ per group). Oils of *Apristurus indicus* (AI), *Centrosymnus crepidater* (CC), *Centrophorus sculpratus* (CS) and *Neohariotta raleighana* (NR) were administered orally at 1.5 g/kg and ibuprofen at 100 mg/kg animal weight.
[a,b,c,d,e] For each hour, percentage inhibition values with different superscripts are significantly different ($P < 0.05$).

standard paracetamol started to show a decline in the inhibitory activity, only CS oil showed a peak inhibitory activity of 69.5% ($P < 0.05$).

DISCUSSION

In the preliminary toxicity studies, 50% mortality was observed at a dosage of 6.2 g/kg body weight. The oils were administered at a safer level of 1.5 g/kg body weight per animal. These oils did not cause any unwanted side effects in the experimental animals, indicating that they were safe for consumption.

Inhibition of acetic acid-induced writhing in mice suggested that the analgesic effect of the oils may be mediated via inhibition of the synthesis and release of prostaglandins (Koster *et al.* 1959). Writhes can be described as a wave of constriction and elongation passing caudally along the abdominal wall with twisting of the trunk and extension of the hind limb in mice (Surender and Mafumdar 1995). The results obtained here showed that the oils at 1.5 g/kg body weight had a higher rate of inhibition than the standard drug paracetamol. Of the four oils analyzed, those belonging to CS, NR and CC species had a better analgesic effect (59, 57 and 52%, respectively) than the standard drug paracetamol (47%) within 90 min from the onset of pain. The hot-plate test also confirmed our findings that the antinociceptive ability of the oils of NR, CS and CC were better off than AI oil.

In the anti-inflammatory study of shark liver oils, a significant inhibition of inflammation began from the second hour, peaked at the third hour and started declining from the forth hour onwards. This is in accordance with a

previously reported study that induction of inflammation involves three distinct phases of release of inflammatory mediators (Surender and Mafumdar 1995). The first phase being the release of histamine and serotonin lasting from the first to the second hour; the second phase being the release of kinins lasting from the second to the third hour while the third phase being the release of prostaglandins and lasting from the third to the fifth hour (Surender and Mafumdar 1995). Thus, it can be inferred that the mechanism through which the oils of CS, NR and CC elicited its effects might be through the inhibition of the synthesis of kinins and prostaglandins, as the oils had been effective at these phases of mediator release. AI oil showed the least inflammation inhibitory effect.

Inflammation is a normal part of the body's immune response to infection or injury. Activated white blood cells secrete a variety of inflammation-promoting compounds or rather inflammatory mediators including cytokines like interleukin-6 and C-reactive proteins (CRP), free radicals and eicosanoids like prostaglandins and leucotrienes to fight germs and to dispose off damaged cells (Ridker *et al.* 2000). It can be assumed that the mechanism of action of liver oils in the present study is via the inhibition of the synthesis of kinins and prostaglandins and this might be through the action on cyclooxygenase (COX) enzyme. COX-2 is responsible for the biosynthesis of prostaglandins under acute inflammatory conditions (Nantel *et al.* 1999). This inducible COX is believed to be the target enzyme for the anti-inflammatory activity of nonsteroidal anti-inflammatory drugs (NSAIDs; Lau *et al.* 1993) which reduce the level of inflammatory mediators and alleviate the pain in the body.

The behavior of shark liver oils from CS, NR, CC and AI species in the descending order of anti-inflammatory activity was 69, 65, 63 and 54%, respectively, as was similar to the standard drug ibuprofen (64%) in the formalin-induced rat-paw edema test, thus confirming their antinociceptive profile and that their action might be similar to that of NSAIDs. Earlier reports have shown that NSAIDs inhibit the activity of COXs (Lau *et al.* 1993) and lower the levels of myeloperoxidase (Faurschou and Borregaard 2003) in the tissues and that they attenuate the pain response in the second phase but not in the first phase of the formalin test in rats.

Antinociceptive and anti-inflammatory components present in the shark liver oils are yet to be explored. Shark liver oils comprise mainly 1-O-alkylglycerols, which constitute about 10–30% of the unsaponifiable matter of the oils (Hallgren and Larsson 1962). These AKGs are indeed responsible for reducing pain or inflammation in the body (Pedrono *et al.* 2004). The exact mechanism by which they function has not been fully understood but it has been proposed that they work by either inhibiting the synthesis, release or action of inflammatory mediators, namely histamine, serotonin and prosta-

glandins that might be involved in inflammation. It has been reported that naturally occurring AKGs have potent biological activities on various cells or systems (Devaraj and Jialal 2000). Shark liver oils also contain high proportions of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (James *et al.* 2003). It has been shown that these long-chain n-3 PUFAs (James *et al.* 2003) lower the incidence of inflammatory diseases such as asthma and arthritis (Shahidi and Senanayake 2006). These dietary FAs are known to reduce the levels of arachidonic acid metabolites and lower the formation of pro-inflammatory 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. Anti-inflammatory effects of EPA and DHA have been studied by several workers (Arita *et al.* 2005; Lukiw *et al.* 2005; Hudert *et al.* 2006). EPA and DHA contained in fish oils provide nutrients needed to build anti-inflammatory prostaglandin series 1 and 3 (Simopoulos 1991). In addition, shark liver oils are rich in antioxidants like vitamin E (Devaraj and Jialal 2000), which reduce inflammation by decreasing CRP levels and by blocking the activity of tumor necrosis factor-alpha series 2-prostaglandins and COXs (James *et al.* 2003). Antioxidants are well-known to alleviate the inflammatory processes mediated by allergic substances. They also curb inflammation by quenching hazardous molecules called free radicals, which stimulate inflammation (Vittala and Newhouse 2004). Sharks inhabiting waters beyond 600 m depth are believed to possess reasonably high content of the HC squalene (Ko *et al.* 2002), yet another antioxidant with potent pharmaceutical values. Its role as an antilipidemic agent (Qureshi *et al.* 1996) and membrane stabilizer has been reported (Sabeena *et al.* 2004).

Pearson's correlation test was used to determine the influence of the HC squalene and AKGs upon anti-inflammation (Table 6). Significant correlations were observed between nonsaponifiable matter (NSM) and anti-inflammation for all the four oils used in the study ($P < 0.01$ for CS, CC and AI, $P < 0.05$ for NR). HC and AKG contributed significantly toward anti-inflammation in CS, NR and AI oils, whereas it was the AKGs which were responsible for the observed anti-inflammatory activity in CC oils ($P < 0.01$). Significant correlations were also observed between HC squalene and antinociception (Table 6): $P < 0.01$ for oils of CC, NR and AI species, $P < 0.05$ for CS species. AKG component of NSM significantly influenced analgesic responses in oils of CC, NR and CS species ($P < 0.05$). Pearson's correlation test proved a significant correlation on the influence of FAs upon anti-inflammation ($P < 0.05$). Saturated FAs and MUFAs showed a negative correlation coefficient with anti-inflammation/antinociception which meant that as their levels increased the anti-inflammatory responses decreased. Positive correlations

TABLE 6.
PEARSON'S CORRELATION COEFFICIENT TEST TO DETERMINE THE INFLUENCE OF VARIOUS LIPID COMPONENTS UPON ANTI-INFLAMMATION AND ANTINOCICEPTION

Oils		Lipid components						
		NSM	HC	AKG	FA	SatFA	MUFA	PUFA
AI	P. corr[a]	1.000†	1.000†	1.000†	1.000*	–0.777	–0.756	0.982
	P. corr[b]	1.000†	0.499	1.000†	1.000*	–0.997	–0.372	0.541
CC	P. corr[a]	1.000†	0.492	1.000†	1.000*	–0.996	–0.365	0.548
	P. corr[b]	1.000*	1.000†	1.000†	1.000†	–0.783	0.849	0.408
CS	P. corr[a]	1.000†	1.000†	1.000†	1.000*	–0.777	–0.756	0.982
	P. corr[b]	0.999*	1.000*	0.497	1.000†	0.406	–0.282	0.596
NR	P. corr[a]	1.000*	1.000†	1.000†	1.000*	–0.780	0.851	0.404
	P. corr[b]	1.000†	0.499	1.000†	1.000*	–0.997	–0.372	0.541

* Correlation is significant at the 0.05 level (2-tailed).
† Correlation is significant at the 0.01 level (2-tailed).
P.corr[a], Pearson's correlation coefficient with respect to anti-inflammation.
P.corr[b], Pearson's correlation coefficient with respect to antinociception.
NSM, nonsaponifiable matter; HC, hydrocarbons; AKG, alkylglycerols; FA, fatty acid; SatFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

were observed between levels of PUFA and anti-inflammation, thus confirming their roles in lowering inflammation (Table 6). The cumulative effects of the various lipid components (squalene, AKGs and PUFAs) studied are indeed responsible for the observed anti-inflammatory and antinociceptive effects of the extracted shark liver oils (Ko *et al.* 2002; Pedrono *et al.* 2004; Arita *et al.* 2005).

The results of the present investigation indicated that shark liver oils belonging to CS, NR and CC species showed a better analgesic and anti-inflammatory profile than that of AI oil. We propose that the high NSM content to be responsible for the observed findings. The liver oils of NR, CS and CC contained a high fraction of NSM (average 70%); the AKGs, HCs and vitamin E could play a major role in lowering the incidence of inflammatory diseases by blocking the activity of prostaglandins and leukotrienes. AI oil recorded the lowest fraction of NSM (25%) and hence exhibited lower effects.

In conclusion, liver oils extracted from the sharks, namely CS, NR, CC and AI, possessed excellent anti-inflammatory and peripheral antinociceptive effects, contributing to its use in the treatment of arthritis and other inflammatory disorders. AKGs, long-chain PUFAs, vitamin E and squalene present in shark liver oils play a major role in reducing the level of inflammatory mediators during an inflammation. The bioactive potentials of marine lipids from creatures inhabiting the Indian EEZ and the ability of these oils to interfere with the inflammatory mediators deserve further investigation.

NOMENCLATURE

AI	*Apristurus indicus*
CC	*Centrosymnus crepidater*
CS	*Centrophorus scalpratus*
NR	*Neohariotta raleighana*
FORV	Fisheries Oceanographic Research Vessel
NSM	nonsaponifiable matter
DMSO	dimethyl sulfoxide
LD	lethal dose
AKG	alkylglycerol
CRP	C-reactive proteins
NSAIDs	nonsteroidal anti-inflammatory drugs
COX	cyclooxygenase
PUFA	polyunsaturated fatty acid
GLA	gamma linolenic acid
EPA	eicosapentaenoic acid
DHA	docosahexaenoic acid

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