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Protective Effects of *Echinorhinus brucus* Liver Oil against Induced Inflammation and Ulceration in Rats

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Abstract

Anti-inflammatory and anti-ulcer activities of Bramble shark (*Echinorhinus brucus)* liver oil were examined in rats. The oil showed significant proportion of n-3 polyunsaturated fatty acids (PUFAs), the percentages of EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) being 16 and 18% respectively. The study also revealed that liver oil had a very favourable n3:n6 ratio of 4.7. Oral administration of shark liver oil at 1g kg^{-1} concentration significantly attenuated the formalin-induced paw edema in experimental rats. It exerted potent anti-ulcer effect against acid-ethanol mixture-mediated lesion formation in the rat gastric mucosa.

Keywords: Bramble shark, liver oil, anti-inflammation, anti-ulcer

Introduction

Inflammation is body's protective response to microbial infection and injury. But this protective response results in adverse reactions like serum sickness and immunological disorders and is mediated by arachidonic acid derivatives such as prostaglandins and thromboxanes. Inflammation normally occurs in response to local disruption to tissues after injury, however it is also seen in conditions such as Alzheimer's disease (Pasinetti & Eberstein, 1996) and cancer (Vane, 2000). Non-steroidal anti-inflammatory drugs (NSAIDs) are generally administered against inflammatory disorders and the side effects of the currently available anti-inflammatory drugs pose a major problem during their clinical use (Gurkirpal, 1998). Therefore there is a need for newer and more powerful anti-inflammatory drugs with minimum side effects. Shark liver oil has proven anti-inflammatory activity (Reddy & Patrick, 2009).

Peptic ulcer is a frequently encountered gastric disease whose etiology is not clear. It is proven that many factors such as pepsin, bile salts, *Helicobacter pylori* and inflammatory mediators like prostaglandins play a vital role in prognosis of this disease (Hoogerwer & Pasricha, 2001; Goel & Bhattacharya, 1991). Treatment of ulcer involves the administration of antacids, protective agents of mucosal tissues and elimination of infective bacteria, *H. pylori* (Award, 2013). Significant side effects are commonly seen due to repeated use of synthetic anti-ulcer agents. Many herbal products and oils are reportedly effective against peptic ulcer and have negligible side effects (Alharbi, 1995; Bafna & Balaraman, 2005).

Fish oils which are a major source of n-3 PUFA and other non saponifiable compounds have been reportedly found to be effective against adverse inflammatory response (Calder, 2006). Shark liver oil has been used for over 40 years as both therapeutic and preventive agent (Daniel et al. 1997). The main ingredients in shark liver oil are alkylglycerols, squalene, vitamins and long chain PUFA. Beneficial health effects of these compounds are well demonstrated and include prevention of number of diseases such as coronary heart diseases, cancer, arthritis, bacterial, viral and fungal infections, allergic reactions, autoimmune disorders, adjuvant therapy and treatment of neoplastic disorders and as immune booster in infectious diseases (Schacky & William, 2003; Pugliese, 1998). Oil of *Dasyatis brevis* and *Gymnura marmorata* contain comparatively high amount of PUFA, n-3 PUFA being dominant among fatty acids (Navarro-Garcia

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et al., 2000; Nechet et al., 2007). Generally, shark liver oils are rich in bio-active compounds but they remain unexplored with regard to their anti-ulcer and anti-inflammatory activities. Bramble shark (*Echinorhinus brucus*) is one of the two species of deep sea sharks in the family Echinorhinidae. This rarely encountered shark is rich in liver oil and its liver oil is highly valued in countries like South Africa as medicine. The aim of this study was to evaluate anti-ulcer and anti-inflammatory activities of liver oil extracted from bramble shark and to determine its fatty acid composition.

Materials and Methods

Bramble shark (12 kg and 5.5 ft) was caught during the cruise No. 318 (400-600 m depth) of the FORV Sagar Sampada between Mangalore and Kochi on the west coast of India. Deep sea trawl net was used for fishing and the specimen was immediately frozen at -20°C onboard and subsequently brought to the laboratory for further analysis. Liver (3.5 kg) was excised and weighed. Lipid extraction was done as described by Folch & Sloane-Stanley (1957), employing a 2:1 mixture of chloroform-methanol. The oil was stored in amber colored bottles, flushed with nitrogen and stored at -60°C. Wistar strain male albino rats (160-200 g) were used for the experiment. They were housed individually in polyurethane cages under hygienic conditions, maintained at room temperature (28±2°C) and provided food and water ad libitum. Animal experiments were performed as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India and approved by the Institutional Animal Ethics Committee (IAEC).

Formalin and dimethyl sulfoxide (DMSO) were procured from Merck (Darmstadt, Germany) and all other chemicals used were of analytical grade. Ibuprofen, used for animal experiments and standards of fatty acid methyl esters, amino acids, etc were purchased from Sigma Aldrich GmbH (Steinheim, Germany). Fatty acid methyl esters (FAMEs) were analyzed by the method of Sankar et al, (2010). A fraction of the lipid extract was saponified with methanolic NaOH followed by methylation in 14% boron trifluoride in methanol (BF_3/MeOH). Methyl esters of the fatty acids thus obtained were separated by gas chromatography (Thermo Trace GC Ultra) equipped with a Perkin Elmer Elite 225® capillary column (30 m ×0.25 mm × 0.25 μ) and a flame ionization detector. Identification and quantification was done with the help of FAME external standard mixture.

Anti-inflammatory activity was determined, as per Hunskaar et al. (1985) using the formalin-induced rat paw edema test. Percentages of inflammation-inhibition were obtained for each group by calculating the difference in mean paw size from control and expressed as percentage (Owolabi & Omogbai, 2007).

Anti-ulcer activity was determined by the method of Hara & Okabe (1985). Male and female Wistar rats kept in standard laboratory conditions were randomly assigned to four groups consisting of 6 animals each. Group I (positive control) received hydrochloric acid-ethanol (0.6%v/v) to induce ulcer while group II acted as negative control and was fed with regular diet only. Group III was pre-treated with liver oil along with the vehicle (Oil: DMSO = 4:1) at 1.5 g kg^{-1} body weight prior to induction of ulcer. Groups IV was administrated DMSO alone. All the animals were fasted overnight before the induction of ulcer. After 4 h of induction of ulcer, all animal groups underwent surgery as per the procedure of Takeuchi et al. (1976). The stomach was inflated with normal saline and then incised and taken for counting the number of lesions and histopathology.

Fatty acid profile of liver oil and anti-ulcer results were expressed as mean ± SD. Multiple comparisons of the significant ANOVA were carried out using Duncan's multiple comparison tests. A p-value <0.05 was considered as statistically significant. Data were analyzed using SPSS 10.0 for Windows (Kirkpatrick & Feeney, 2003).

Results and Discussion

Fatty acid profile of liver oil extracted from bramble shark had major nutritionally significant fatty acids and they contribute to the bioactivity of the oil (Table 1). Palmitic acid, myristic acid, and stearic acid are the prominent saturated fatty acids. The unsaturated fatty acids oleic acid, linoleic acid, linolenic acid, arachidonic acid, EPA and DHA contribute to the major portion of PUFA (Table. 1). The n-3: n-6 ratio of the oil was observed to be 4.7:1 The fatty acid composition of liver oil from bramble shark was similar to that of shark species from Indian EEZ (Mathen et al. 2008).

Table 1. Fatty acids from liver oil of *Echinorhinus brucus*. Data expressed as a percentage of wet weight. Value represents the mean ± SD

Fatty Acid	(%) in terms of total fatty acids
C14 (Myristic acid)	2.36±0.40
C16 (Palmitic acid)	14.79±0.03
C16:1 (Palmitoleic acid)	3.51±0.01
C17:1 (Heptadecanoic acid)	2.01±0.06
C18:0 (Stearic acid)	8.27±0.20
C18:1n9 (Oleic acid)	12.13±0.11
C18:2n6 (Linoleic acid)	9.24±0.05
C18:3n3 (α Linolenic acid)	0.89±0.09
C18:3n6 (γ Linolenic acid)	2.23±0.13
C20:1 (Eicosenoic acid)	0.55±0.02
C20:3n3 (Eicosatrienoic acid)	5.17±0.10
C20:5n3 (EPA)	16.27±0.22
C22:6n3 (DHA)	18.1±0.99
C23:0 (Tricosanoic acid)	1.13±0.89

Formalin-induced rat paw edema model is sensitive to most clinically effective anti-inflammatory agents (Ruangsang et al. 2010). When formalin is subcutaneously injected into a rat, it forms edema around the injected place, due to increased vascular permeability of the capillary venules in the skin. Substances that antagonize the activity of histamine receptors reduce the area of the edema formed. In this study, the paw size data of control and treated groups of rats is summarized in table 2. Results obtained revealed significant anti-inflammatory effect of *E. brucus* liver oil. A steady increase of paw size was observed for control rats and maximum paw size of 6.31±0.33 mm was observed after 3 h. Compared to control, steady decrease of paw size was observed for liver oil-treated samples. Paw size was minimum (5.25±0.44 mm) for liver oil treated group which is closer to that of Ibuprofen treated sample (4.91±0.25 mm). Table 3 depicts percentage of inhibition of paw size of treated animals. Significant percentage of inhibition 47.8±0.11 was observed for liver oil treated sample. The above result was similar to that of ibuprofen treated sample 48.4±0.21% after 3 h. The analgesic and anti-inflammatory properties of liver oil of four different sharks, namely *Neohariotta raleighana*, *Centrosymnus crepidater*, *Apristurus indicus* and *Centrophorus scalpratus*, captured from the Arabian Sea and the Indian Ocean were reported by Mathen et al. (2008). In comparison to control rats, liver oil-treated animals showed significant reduction of paw size which indicates significant anti-inflammatory effect. There was no significant reduction of paw size for control and DMSO treated samples. As expected ibuprofen-treated groups were superior among all groups with significant decrease in paw size.

Table 3. Anti-inflammatory activity of liver oil extracted from *E. brucus* on formalin induced paw edema in rats. Value represents the mean of six animals ± SD.

Percentage of inhibition (%)	1st h	2nd h	3rd h
Ibuprofen	39.5±0.12	46.5±0.18	48.4±0.21
DMSO	16.6±0.21	25.8±0.22	16.8±0.28
Liver oil	43.5±0.11	48.4±0.10	47.8±0.11
Control	15.8±0.18	29.3±0.15	18.4±0.11

Anti-ulcer effect of shark liver oil was evaluated by comparing the degree of gastric ulceration in treated versus control animals by histopathology (Fig. 1). Oxidative stress is known to play an important role in the pathogenesis of gastric mucosal injury. HCl-ethanol induced gastric lesions are thought to arise

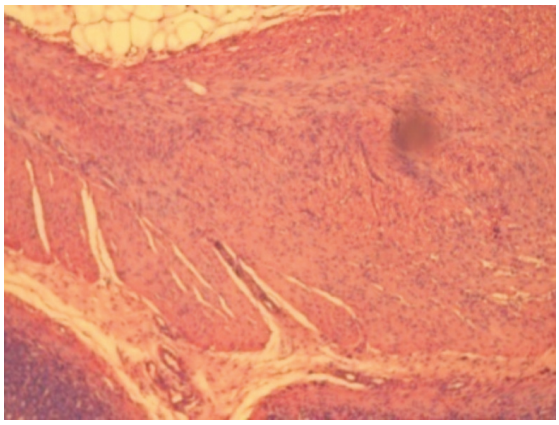
Table 2. Anti-inflammatory effect of shark liver oil from *E. brucus* on formalin induced gastric ulcers in rats. Results are mean±SD of three duplicates of paw size. Values in the row with a different superscript letter (A, B, C) differ significantly ($p<0.05$) with each other.

Paw size (in mm)	Control (I)	Ibuprofen (II)	DMSO (III)	Liver Oil (IV)
Initial	3.15 ± 0.11^{A}	3.28 ± 0.19^{A}	3.47 ± 0.19^{B}	3.60 ± 0.06^{B}
First h	6.82 ± 0.19^{C}	5.50 ± 0.27^{A}	6.53 ± 0.11^{B}	5.67 ± 0.32^{A}
Second h	6.68 ± 0.22^{B}	5.17 ± 0.22^{A}	6.09 ± 0.17^{B}	5.42 ± 0.43^{A}
Third h	6.31 ± 0.33^{C}	4.91 ± 0.25^{A}	6.10 ± 0.41^{B}	5.25 ± 0.44^{A}

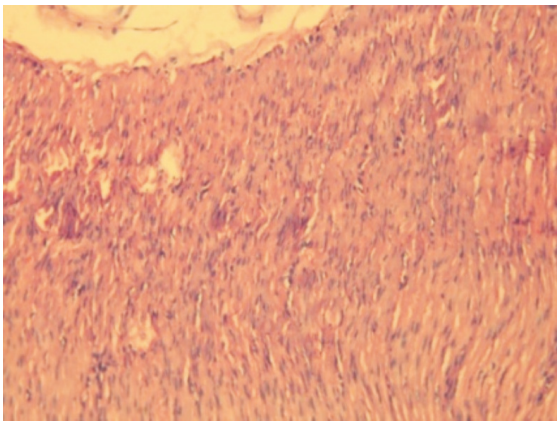
as a result of direct damage of gastric mucosal cells, resulting in the development of free radicals and hyper oxidation of lipid (Jainu & Devi, 2006). Ulcer lesions were noticed in positive control. Reduction of ulcer lesions (Fig. 2) were observed in shark liver oil-treated animal. Severe blood vessel damage and tissue damage with 11-12 lesions was observed for positive control. Whereas reduction of tissue and blood vessels damage was noticed for liver oil-treated sample. The number of lesions has also been reduced to 2-3. Oils of different origin were proven as effective anti-ulcer agents. Plant oils are reportedly known for their anti-ulcer properties (Takayama, 2011; Singh & Manjumdar, 1999). Bioactive compounds present in oils could be the reason behind their antiulcer properties (Peana et al., 2002). The anti-ulcer effect may be due to the presence of bioactive fatty acids and other potent compounds in the fish oil (Alharbi et al., 1995)

Histopathology of the gastric mucosa of (A) Shark liver oil and DMSO (Dimethyl sulfoxide) treated rats in which ulcer was induced by HCl -Ethanol (B) Normal control rats: without ulcer induction (C) DMSO treated rats D) Positive control rats: ulcer induced by HCl-Ethanol showing cell necrosis

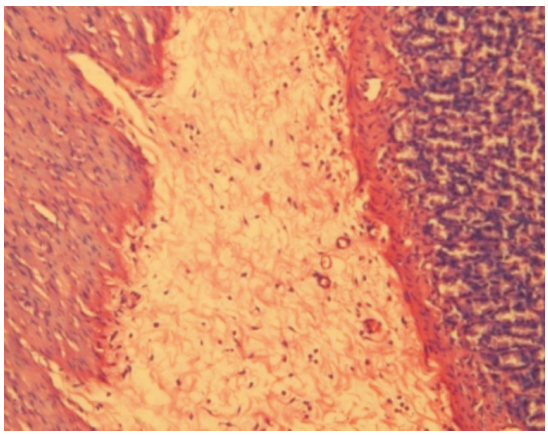
Gastric mucosa of (A) control rats; ulcer induced by HCl-Ethanol showing the gastric mucosal damage (b) Negative control rats; without ulcer induction (c) Shark liver oil and DMSO treated rats; ulcer induced by HCl-Ethanol and D) DMSO treated rats; ulcer induced by HCl-Ethanol



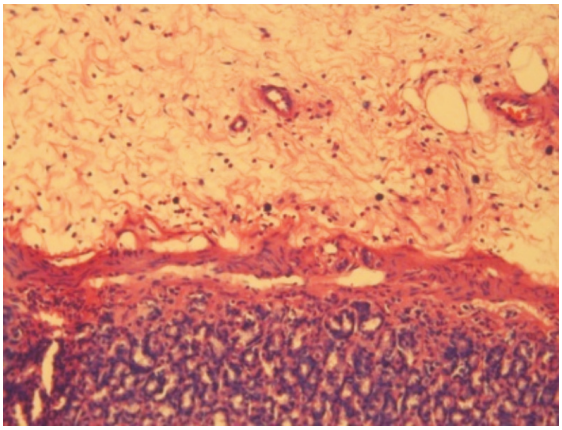
A) Oil with DMSO



B) Normal control



C) DMSO



D) Positive Control

Fig. 1. Anti-ulcer activity of liver oil extracted from bramble shark on gastric mucosa: Histopathological section.

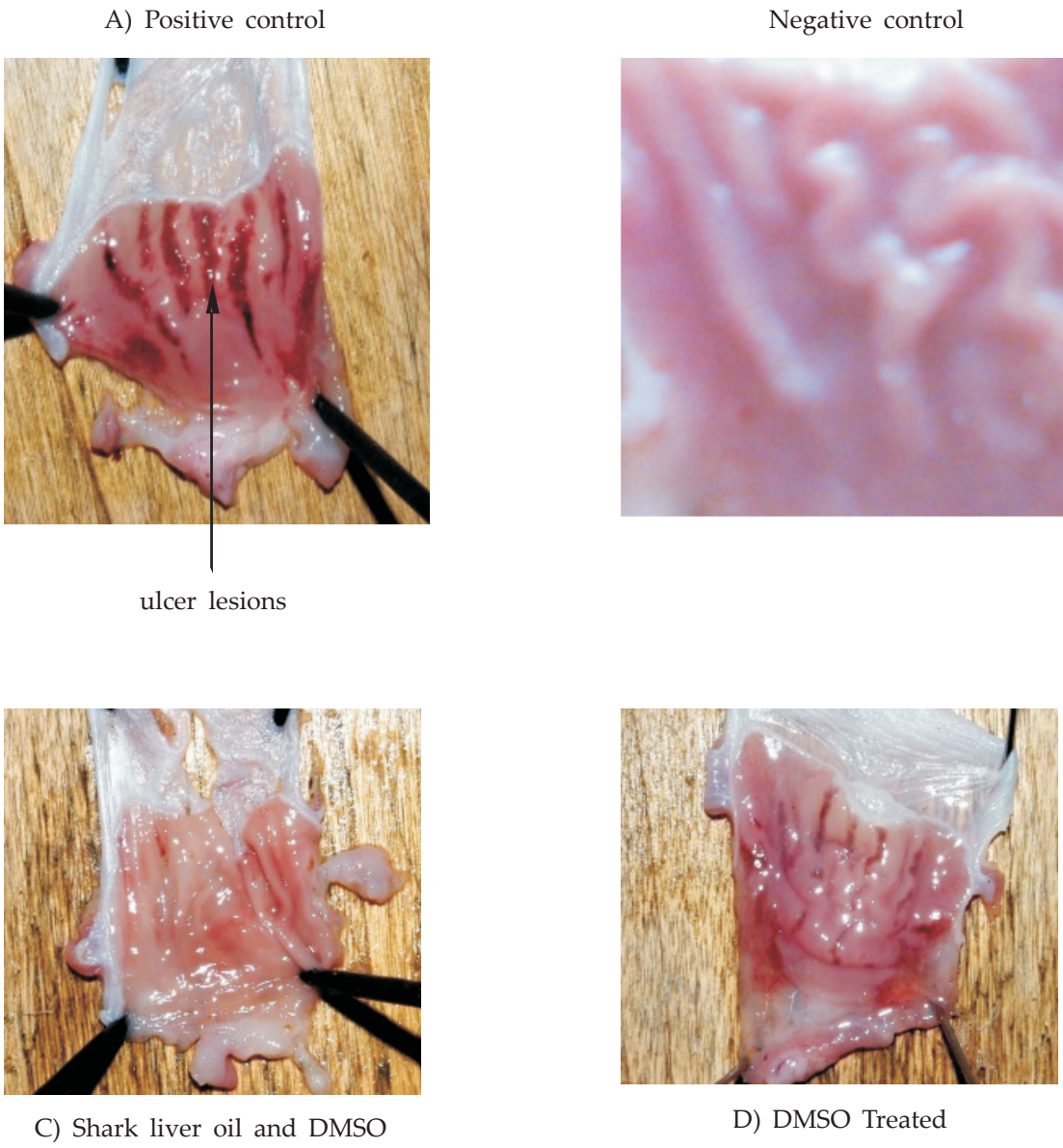


Fig. 2. Anti-ulcer activity of liver oil extracted from bramble shark on gastric mucosa

Liver oil extracted from Bramble shark (*E. brucus*) has proven to be anti-inflammatory and anti-ulcerogenic. It can be a potential source of medicine against these diseases and consumed as a nutraceutical. So liver oil from Bramble shark has the potential to be used for nutraceutical development.

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