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PHARMACOLOGICALLY ACTIVE FATTY ACIDS OF FIDDLER CRAB *Uca acuta acuta* (Simpson)

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ABSTRACT

The fatty acid compositions of the body flesh, big chela flesh and hepatopancreas of the selected detritivore fiddler crab *Uca acuta acuta* (Simpson) have been studied by gas liquid chromatography and other chromatographic techniques. Altogether 28 components were detected and estimated. Major fatty acids recorded were palmitic (16:0), stearic (18:0), oleic (18:1ω9), linoleic (18:2ω6), arachidonic (20:4ω3), eicosapentaenoic (20:5ω3) and docosahexaenoic (22:6ω3). Of the polyunsaturated fatty acids, considerably higherlevels of pharmacologically active fatty acids, viz. eicosapentaenoic and Docosahexaenoic acids were recorded. Such findings suggests that the species studied is also a potential source of pharmacologically active fatty acids, particularly those belonging to the –ω3 series.

Key words: Fatty acid, Fiddler crab, EPA&DHA

1. INTRODUCTION

Biochemical studies on fiddler crab *Uca acuta acuta* (Simpson) belongs to the family Ocypodidae of the class Malacostraca is necessary for evaluating its nutritional value as well as its possibility of future usage as natural sources for biologically active components. Lipids and particularly the polyunsaturated fatty acids (PUFA) have long been known to be essential for the maintenance of good health of any individual. Omega-3 [(n-3)] long-chain PUFA, including EPA and DHA, are dietary fats with an array of health benefits (Su, K.P et al, 2008). They are incorporated in many parts of the body including cell membranes (Lazzarin, N. et al. 2009) and play a role in anti-inflammatory processes and in the viscosity of cell membranes (Smith, G.I et al.2011; Conquer, J.A. et al. 2000). EPA and DHA are essential for proper fetal development and healthy aging (Dunstan, J.A. et al .2007). DHA is a key component of all cell membranes and is found in abundance in the brain and retina (Krauss-Etschmann, S. et al.2007). EPA and DHA are also the precursors of several metabolites that are potent lipid mediators, considered by many investigators to be beneficial in the prevention or treatment of several diseases (Serhan, C.N. et al.2008). Interests on the effects of fish oil on human health have been accelerated in the current years with the studies carried out by researchers.(Dyrberg et al,1975). Such studies have reported on the variety of heart diseases among Greenland Eskimos and their relationships with the consumption of lipids high in ω3 PUFA. Lipids are necessary as a reservoir of energy, the transport of fat soluble vitamins and the source of certain essential fatty acids. However, studies have been carried out on the effects of certain fatty acids, especially PUFA(Pigott and Tucker 1987). The fractional components of particular interest in fish oils involves the omega-3 fatty acids, especially

eicosapentaenoic acid (20:5ω3,EPA) and docosahexaenoic acids (22:6ω3,DHA). Studies have shown that dietary EPA prevents medical disorders in heart and circulatory diseases (Simopoulos 1991;Connor and Connor 1997), reverses impairment of endothelium-dependent relaxation(Chin and Dart1994), inhibits platelet activation(Hay et al.1982), reduces monocyte attachment to arterial endothelium and suppresses release of toxic, mitogenic and prothrombotic agents(Kim et al.1990), DHA is also effective in skin disorders, aids brain development and forms a good part of the retina of the eye(Lee et al. 1985). Previous studies made on the fatty acids of tiger prawn *penaeus monodon* (Fabricius) by a group of researchers (Bandyopadhyay C. et al, 1993), has shown a rich source of both eicosapentaenoic acid (EPA) and docosahexaenoic acids (DHA). Nutritional evaluation of liver and body flesh lipids of ray fish, *Dasyatis bleekeri* (Blyth) has also shown that liver and flesh of ray fish, *Dasyatis bleekeri* is a good source of marine oils as well as -ω3 PUFA and they would be suitable for inclusion in the formulation of highly unsaturated diets (Pal D. et al, 1999). In the present study, efforts have been made to determine various lipid components in the body of the fiddler crab *Uca acuta acuta,* a very common and abundant macro-benthic intertidal faunal component in the coastal mangrove estuarine complex of West Bengal(Chakraborty S.K.,2011) from the selected study area of Midnapur (East) costal belt, West Bengal, in between the Latitude 21°47′(N) and Longitudes 87°45′(E) with special reference to the fatty acid profile of the lipid.

2. MATERIALS AND METHODS

The studied fiddler crabs, *Uca acuta acuta* were collected from the coastal belt of Purusattampur (Dadanpatrabar), Midnapur(East), West Bengal (Latitude 21°47′N and longitudes 87°45′E) . Different body parts of the collected specimens such as i) Body muscles, ii) Largest Chela Flesh and iii) Hepatopancreaus were dissected out of the body and were immediately frozen and stored at -20°c until analyzed.

2.1 Extraction of Lipids

The total lipids were extracted from the samples following the method of Bligh and Dyer (1959) using methanol chloroform (2:1,v/v), methanol-chloroform – water (2:1:0.8, v/v/v), and then again with the first solvent system. Samples were grounded with the solvent, in a high speed homogenizer, filtered and residues were extracted with, the next solvent system. The process was repeated. Finally, the three extracts were pooled, diluted with water and layer was allowed to separate in a separatory funnel. The chloroform layer at the bottom was withdrawn and dried over anhydrous sodium sulphate in a freezer. The chloroform solution of lipid was evaporated under vacuum, redissolved in distilled n-hexane and kept at -20°C for future use. BHT (Butylated Hydroxy Toluene) was added at a level of 100mg/L to the solvent as antioxidant. After dilution of the pooled extracts, a heavy white precipitate appeared at the junction of the two layers which were kept for further analysis.

2.2 Preparation of Methyl Esters of Fatty Acids

Total lipids were transformed into methyl esters by trans-methylation. The samples were dissolved in anhydrous methanol containing concentrated Sulfuric acid (1.0%, v/v) and the mixture were refluxed (Christie,1982) for 2 hours. Methanol was evaporated to a small volume and cooled. Distilled water was added to the cooled mixture and the methy1 esters of Fatty acids were extracted 3 times with aliquots of diethy1 ether. The ethereal extracts were pooled and dried over anhydrous sodium sulfate, filtered, vacuum dried, dissolved in n-hexane and kept in a freezer for further use.

2.3 Purification of Fatty Acids Methyl Esters By Thin Layer Chromatography (TLC)

Fatty acid methyl esters were purified (Mangold, 1969) by TLC using a solvent system of n-hexane-diethyl ether (90:10,v/v) . A standard methyl ester was also run on the same plate in a separate lane. The location of methyl ester bands corresponding to the standard were marked after placing the TLC plate in an iodine vapour chamber and then scrapped off the methyl ester band from the plate. Methy1 esters were recovered by extracting the recovered bands in a mini glass column with chloroform, the later was evaporated and the Methy1 esters were kept in n-hexane in a freezer, till analyzed by GLC.

2.4 Gas Liquid Chromatography (GLC)

GLC of fatty acid Methy1 esters were done on a Chemito 1000 instrument, equipped with Flame

lonization Detector (FID). Quantitation was done by computer using specific clarity lite software.

2.5 Analysis of Fatty Acid Methy1 Ester (FAME)

GLC of FAME was done on a BPX-70 megabore capillary coloumn of 30 mt length and 0.53 mm i.d. obtained from SGE, Australia. Oven temperature was programmed from 150°C - 240°C with a rate of 8°C/min. Initial and final times were kept isotheremal for 1 minute and 20 minutes, respectively. Injection port and detector temperatures were 250°C and 300°C, respectively. Nitrogen gas was used as carrier gas, its flow being 6.32 ml/min. Identification of Fatty acids was done by comparing their retention times with those of standards, chromatographed under identical operational conditional of GLC. Confirmation of fatty acids was also done by using the FAME of Cod liver oil fatty acids, as suggested (Ackman and Burger,1965 and Ackman et al. 1963).

3. RESULTS AND DISCUSSION

The present investigation has revealed that the body flesh of *Uca acuta acuta* contains a considerable amount of both eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The occurrence of considerably higher levels of EPA and DHA in the body flesh is common in detritivorous benthic animals of Sundarbans estuarine complex (Misra et al. 1983). About 28 fatty acids have been found to occur in the body flesh, big chela flesh and hepatopancreas of *Uca acuta acuta* (Table-1& Fig-1,2&3).

Table 1

Faty acid compositions of Total Lipids (TL) from Uca acuta acuta (Fiddler Crab) as determined by GLC of methyl esters (% w/w of each component in total fatty acids).

Components[a]	Body Flesh	Big Chela Flesh	Hepato Pancreas
13:0		1.0	0.2
14:0	2.2	4.3	6.9
14:1	2.3		0.1
15:0	0.7	4.5	0.4
15:1	21.6		0.3
16:0	9.9	18.7	13.6
16:1	0.2	8.0	10.3
16:2	2.5		2.5
17:0	0.6	1.3	3.0
17:1	9.4	2.6	3.9
18:0	6.3	12.4	10.7
18:1ω9	2.9	7.2	9.6
18:2ω6	0.5	4.7	4.4
18:3ω6	0.3	0.4	1.2
18:3ω3	0.2	0.3	1.0
20:3ω3	0.1	1.5	1.0
20:4ω6	0.4		0.7
22:0	0.1	0.1	0.3
22:1	6.2	1.0	0.3
20:4ω3	0.1	8.4	9.8
22:4ω6	22.3	0.1	0.2
22:5ω3	0.1	14.3	13.3
21:5ω3			0.2
22:5ω6			0.007
24:0	0.7	0.5	1.0
24:1	0.4	1.0	0.7
22:5ω3	0.1	0.2	0.2
22.6ω	9.3	7.0	4.8

[a] *First and second figures represent, carbon chain length: number of double bonds. The -ω values represent the methyl end chain from the center of double bond furthest removed from the carboxyl end.*

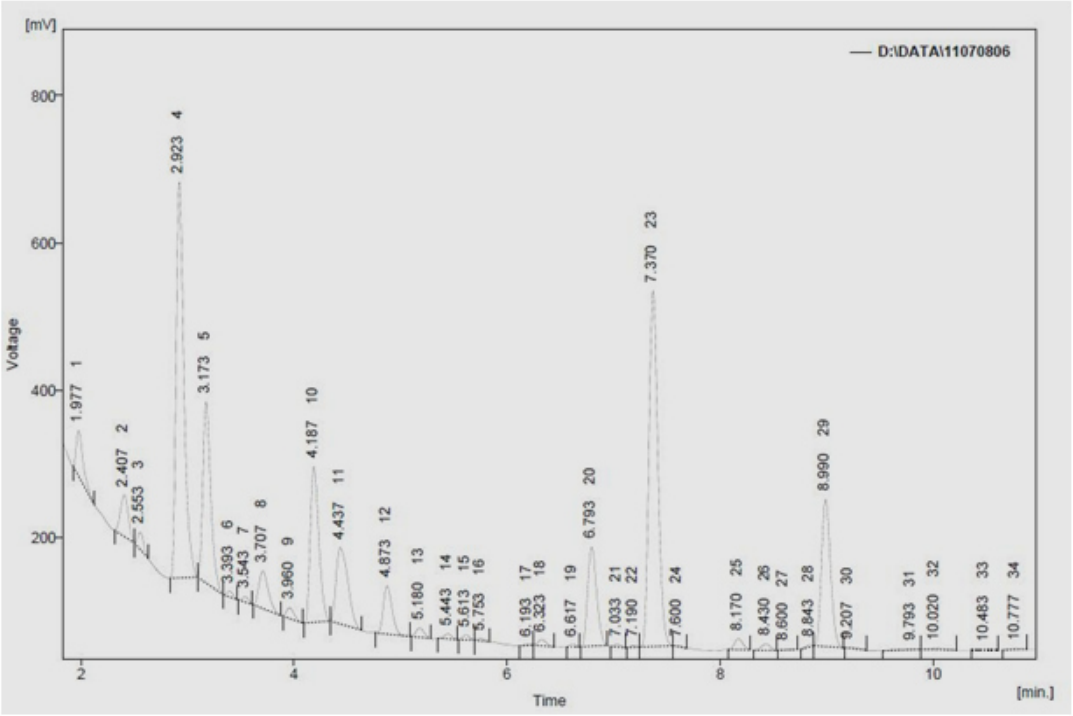

Figure1

GLC tracing of the FAME of the TL of body flesh, of the crab, Uca acuta acuta. GLC column used was BPX-70 (Polar column) Megabore column (30 meters length x 0.530 mm dia).

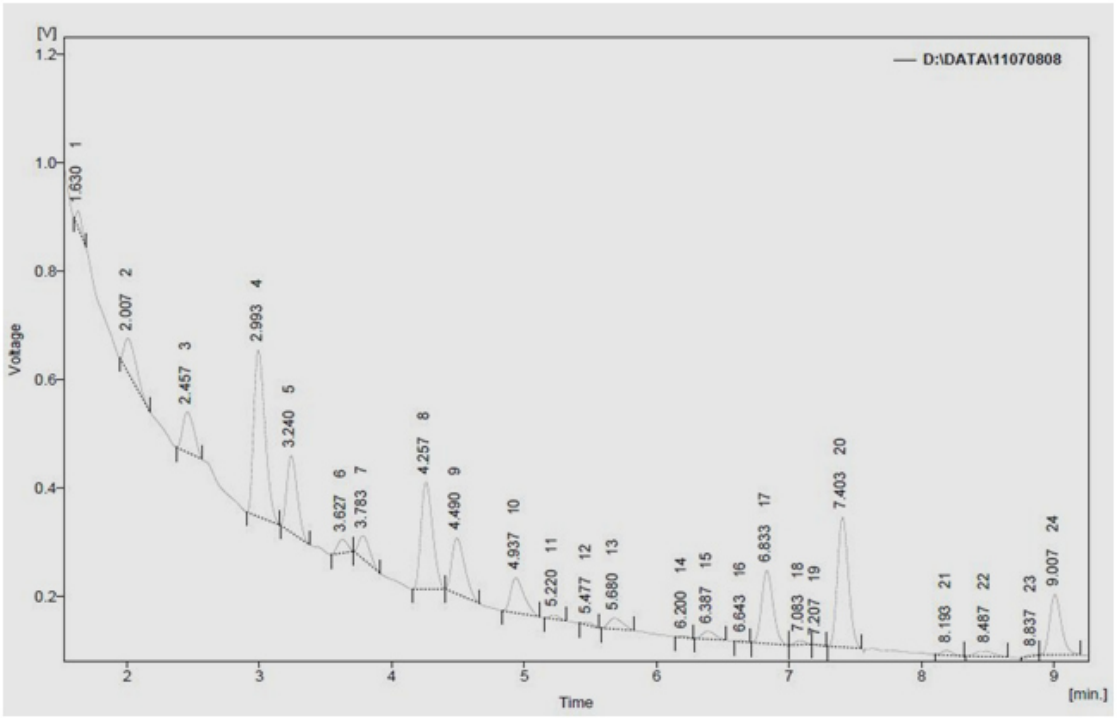

Figure 2

GLC tracing of the FAME of the TL of big chela flesh, of the crab,Uca acuta acuta. GLC column used was BPX-70 (Polar column) Megabore column (30 meters length x 0.530 mm dia).

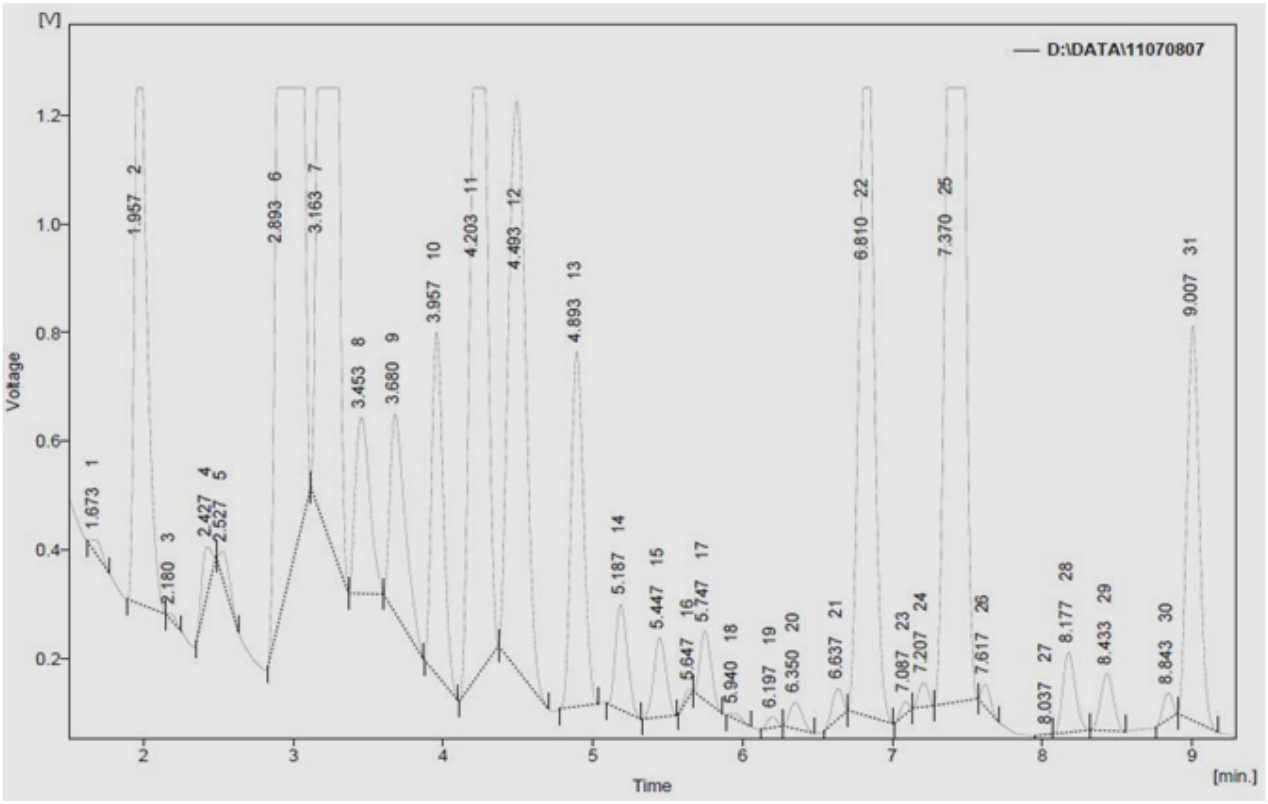

Figure 3

GLC tracing tracing of the FAME of the TL of hepatopancreas, of the crab, Uca acuta acuta. GLC column used was BPX-70 (Polar column) Megabore column (30 meters length x 0.530 mm dia).

Of the saturated fatty acids, the highest amount was represented by palmitic acid (21.6%) in the body flesh and 18.7% in big chela flesh but such fatty acid was 13.6 % in the hepatopancreas as obtained. .Among the unsaturated fatty acids, the major component was eicosapentaenoic acid (22.3%) in the body flesh, 14.3% in big chela flesh and it was 13.3% in the hepatopancreas . The EPA is the major bioactive fatty acid. Of the other polyenoic acids, special mention is to be made on DHA, which was found only at 9.3% in body flesh, 7.0% in big chela flesh and 4.8% in the hepatopancreas .Omega-3 fatty acids, the principal building blocks of marine fish oils, have a number of health –enhancing properties. Already well known for their ability to protect against heart disease, cancer, and diabetes,(Assisi, A. et al.2006) the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) may be highly effective in preventing and managing depression and cognitive decline, according to a growing body of evidence(Raeder, M.B. et al.2006; Frangou, S. et al.2006 and Peet, M. et al.2002). Low dietary intake of beneficial omega-3 fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), is linked to depressed mood, hostility, and impulsive behavior(Ross, B.M. et al.2007; Conklin, S.M. et al 2007 and Conklin, S.M. et al.2007). High intake of EPA and DHA is associated with increased gray matter volume in brain regions controlling depression and mood(Conklin, S.M. et al.2007). These fatty acids, especially the EPA, in the diet reduce concentrations of cholesterol and triglycerides of the plasma by lowering the rate of the synthesis of low density lipoprotein , which act as carriers of triglycerides and cholesterol (Illingworth, 1984), by the liver and vascular tissues. Thus it is suggested that adult patients with circulatory and other symptoms can be treated medically if EPA is taken regularly in fish-oil capsule form and various heart diseases can be prevented (Ackman, 1986; Ackman, 1988). Similar studies with DHA indicate that it is effective in skin disorders, relieves inflammatory conditions, aids brain development and also forms a good part of the retina of the eye.

4. CONCLUSION

In the present study, it has been established that, considerably higher levels of EPA and DHA have been found in the various tissues of *Uca acuta acuta*, particularly in the body flesh of the animals which have various therapeutic effects.

5. ACKNOWLEDGEMENT

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