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RESEARCH ARTICLE

SEASONAL TOXICOLOGICAL PROFILE OF THREE PUFFER FISHES COLLECTED ALONG DIGHA COASTAL BELT, WEST BENGAL, INDIA

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ABSTRACT

Puffer fish intoxication is extremely frequent in countries like Japan, China, Taiwan, Hong Kong, Philippines, Thailand, Bangladesh, India etc. In Digha coastal belt of West Bengal, India, three puffer fishes are mostly abundant viz. *Lagocephalus lunaris* (Bloch and Schneider, 1801); *Takifugu oblongus* (Bloch, 1786); *Tetraodon fluviatilis* (Hamilton, 1822). These three puffer fish species are studied for toxicological characterization. In the present study, Gonado-Somatic Index (GSI), Hepato-Somatic Index (HSI) and Lethal Dose 50 (LD_{50}) is observed. To understand the toxicity level in three different seasons, LD_{50} has been done by using *Labeo rohita* as test animal. Isolated toxin of puffer fishes was injected intramuscularly to *Labeo rohita* and toxicity was measured in three different seasons that are pre-monsoon, monsoon and post-monsoon. It is observed that in three puffer fishes toxicity level in monsoon season is highest as compared to other seasons. Gonad and liver tissue is more or less equally toxic in three puffer fishes. More lethal potency of gonad has a correlation with spawning and egg maturity.

INTRODUCTION

Toxicity is prevalent among marine organisms like algae, jellyfish, ascidians, bryozoans, sea anemones, corals, sponges, echinoderms, molluscs, crustaceans and fishes (Hay, 1996; Saha *et al.,* 2015). Toxicity of puffer fishes are the best known of all types of fish intoxications and has been documented from ancient times. Puffer fishes are known as second most poisonous vertebrate in the world after "Golden Poison Frog" (Keiichi *et al*, 1998). Toxicity of puffer fishes is due to occurrence of Tetrodotoxin (TTX) in tissues like liver, skin and gonads (Chau *et al*., 2011; Monaliza and Samsur, 2011). TTX is chiefly a powerful neurotoxin, which can block voltage-gated sodium channels on the surface of nerve membranes (Narahashi, 2001). Puffer fish intoxication is predominant in Japan but a number of cases have been reported in many other Asian countries such as China, Taiwan, Hong Kong, Philippines, Thailand, Bangladesh, India as well as other continents (Arakawa *et al,* 2010; Kungsuwan, 1993). 29 genera of family Tetraodontidae has been recognized (R. Froese and D. Pauly, 2008; Froese and Pauly, 2014) and about 120 species of puffer fishes are reported from the tropical seas (Sabrah *et al,* 2006). In coastal region of West Bengal especially Digha coastal belt 7 puffer fish species have been reported (Nath and Kundu, 2017). Toxicological investigations on puffer fishes revealed that the ovaries showed maximum lethal potency in their spawning season (Ghosh *et al,* 2004). Gonado-Somatic Index (GSI) and Hepato- somatic Index (HSI) data suggest that the amplified toxin in ovary is the product of embryos (K. Matsumura, 1998). The majority of fish intoxication is due to puffer fish toxin along the coasts of Asia (Ahasan *et al,* 2004; Wu *et al,* 2005; Hwang *et al*, 2002). In Digha coastal belt of West Bengal, India, 3 puffer fishes are most abundant viz. *Lagocephalus lunaris* (Bloch and Schneider, 1801); *Takifugu oblongus* (Bloch, 1786); *Tetraodon fluviatilis* (Hamilton, 1822) (Nath and Kundu, 2017). These three puffer fish species are studied for toxicological characterization. In the present study, to understand the toxicity level in three different seasons, toxicity studies based on LD_{50} by using the test organism (*Labeo rohita*) has been done. Isolated toxin of puffer fishes was introduced to *Labeo rohita* and toxicity was measured.

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MATERIALS AND METHODS

Sample Collection

Collection of Puffer fishes were made from the coastal region of Digha (between 21°32´N to 21°45´N latitude and 87°32´E to 87°50´E longitude), in Purba Medinipur District, West Bengal, east coast of India. Trawling has been done from three different fish landing centers (Fig 1) of this coastal belt which are tabulated below (Table 1). Samples had been collected for three years (January, 2013 - January, 2016). After collection the samples were stored in different ice boxes according to their length and weight. Then the samples were transported to the laboratory and immediately washed, kept in sterile saline water and stored at -20°C until use. The catch was segregated into three distinct seasons (Table 2).

Table 1. Three Trawling stations with GPS Location where samples were collected periodically from Digha coast during this survey

Sl. No.	Station Name	GPS Location
Station 1	Digha mohona	21°37´N latitude and 87°32´E longitude
Station 2	Sankarpur	21°38´N latitude and 87°34´E longitude
Station 3	Soula	21°44´N latitude and 87°50´E longitude

Crude Toxin Preparation

3 most abundant puffer fishes are studied for toxicological characterization viz. *Lagocephalus lunaris* (Bloch and Schneider, 1801); *Takifugu oblongus* (Bloch, 1786); *Tetraodon fluviatilis* (Hamilton, 1822). The puffer fishes were brought to the laboratory and immediately washed, kept in sterile saline water and stored at -20° C until further use. By random sampling 35 puffer fish specimens of each species were chosen for toxicity assessment in each season. For toxicological experiment the extraction was done according to the methodology of Khora, 1991. First of all livers, skin of belly region and ovaries of each different puffer fishes were dissected out and kept in vacuum sealed container under -20 °C. The total weight of each tissue was recorded before being crushed into small portion at about 10g. 2.5 volumes of 0.1%

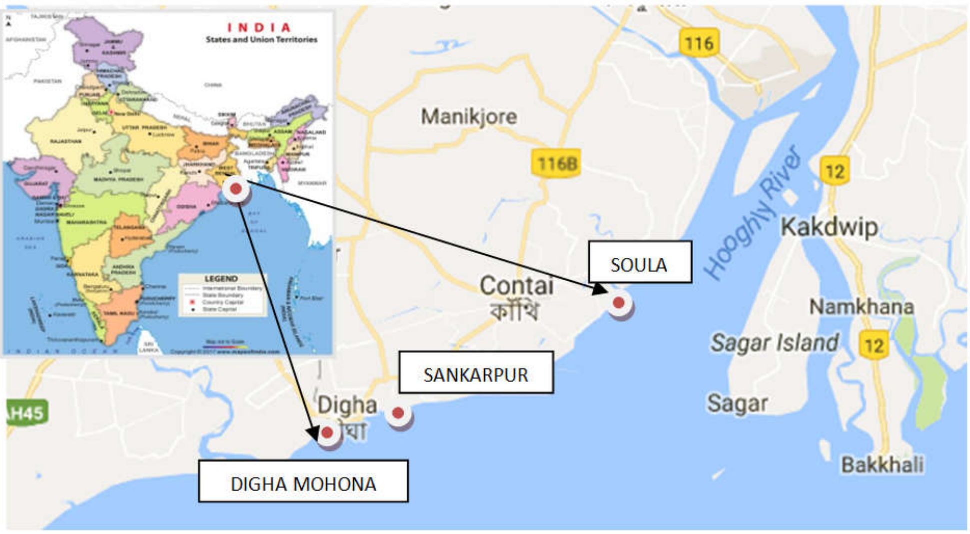

Figure 1. Location map of study region and three fish landing centers (Station 1 - Digha mohona, Station 2 - Sankarpur, Station 3 - Soula) along Digha coastal region, West Bengal, east coast of India

Table 2. Catch seasons of puffer fish species

Season	Month	Temperature	Rainfall
Pre Monsoon	April - June	29°C - 35°C	0.8 – 1.8 Cm
Monsoon	July - September	26°C - 33°C	8.5 – 10.8 Cm
Post Monsoon	October - December	19°C - 25°C	0.7 – 3.6 Cm

Study of GSI & HSI

Sex and maturity were determined macroscopically and the gonad weights and liver weights were recorded to the nearest 0.01 g. The Hepato Somatic Index (HSI) of the fish was determined by the use of equation cited by R. I. Welcomme, 2001.

$$HSI = \frac{\text{Weight of liver} \times 100}{\text{Weight of body}}$$

Similarly the gonadosomatic index (GSI) of the male fish (i.e. the weight of testis) and female fish (i.e. the weight of ovaries) were determined by equation cited by R. I. Welcomme, 2001.

$$GSI = \frac{\text{Weight of gonad} \times 100}{\text{Weight of body}}$$

acetic acid was added with the crushed small pieces of sample which was treated with boiled water for 10 minutes. The slurry was centrifuged at about 3000 rpm for 10 minutes to take the supernatant. This course of action was repeated for three times to obtain 5 volumes of TTX from the sample. Then all the three supernatants were mixed together (Veeruraj *et al,* 2016; Saha *et al,* 2015; Khora, 1991).

Experimental Animals

For toxicity assay, *Labeo rohita* (Rahu) a fresh water fish were collected from the ponds and rivers of Purba Medinipore. A total of 300 fishes that were approximately 7±1.5 grams in weight and 9±1.3 cm in size were brought to the laboratory three week prior to the performance of experiment for acclimatization. They were reared in 60 x 30 cm aquarium tanks with aerator in each of the tanks with 10 fishes. They were fed with artificial pellets.

Experimental Design

After acclimatizing *Labeo rohita* (Rahu) to the laboratory conditions for three week, the liver, ovary and skin extracts of those three puffer fishes as well as the saline solution were injected intra-muscularly to *Labeo rohita*. Fishes in the control tanks were injected with 0.1, 0.2 and 0.3 ml of saline solution (0.9% NaCl) per gram body weight respectively.

Fig 2A: ***Lagocephalus lunaris*** **(Bloch and Schneider, 1801)**

Fig 2B: ***Takifugu oblongus*** **(Bloch, 1786)**

Fig 2C: ***Tetraodon fluviatilis*** **(Hamilton, 1822)**

Table 3. Anatomical Distribution of toxicity (LD_{50}), HSI (hepato-somatic index), GSI (gonado-somatic index) of *Lagocephalus lunaris* (Bloch and Schneider, 1801), *Takifugu oblongus* (Bloch, 1786), *Tetraodon fluviatilis* (Hamilton, 1822) in three different seasons

Puffer fish Species	Season	Body weight (gm)	Length (cm)	M/F Ratio	HSI	GSI	LD50 Value (ml/gm body weight)		
							Liver extract	Gonad Extract	Skin extract
Lagocephalus lunaris	Pre monsoon (N =35)	138.3 ± 9.4	19.33 ± 1.2	1 : 1	3.91 ± 0.9	1.13 ± 0.4	0.25 ± 0.05	0.20 ± 0.01	0.40 ± 0.07
	Monsoon (N =35)	166.9 ± 10.2	18.87 ± 2.7	1 : 2	8.55 ± 1.5	5.81 ± 1.9	0.15 ± 0.01	0.15 ± 0.07	0.35 ± 0.09
	Post monsoon (N =35)	181.2 ± 13.7	19.99 ± 1.5	2 : 3	6.89 ± 0.8	4.28 ± 2.2	0.35 ± 0.09	0.30 ± 0.05	0.45 ± 0.04
Takifugu oblongus	Pre monsoon (N =35)	280.9 ± 10.1	15.62 ± 5.9	3:4	9.74 ± 2.6	0.44 ± 0.1	0.20 ± 0.03	0.25 ± 0.02	0.55 ± 0.10
	Monsoon (N =35)	310.7 ± 21.2	14.88 ± 4.3	1:2	12.29 ± 3.2	4.12 ± 1.3	0.10 ± 0.01	0.15 ± 0.03	0.25 ± 0.05
	Post monsoon (N =35)	366.3 ± 31.7	15.01 ± 6.9	1:3	10.11 ± 2.7	2.9 ± 0.4	0.35 ± 0.06	0.45 ± 0.15	0.15 ± 0.15
Tetraodon fluviatilis	Pre monsoon (N =35)	130.9 ± 12.2	9.57 ± 8.7	1:1	7.61 ± 1.2	0.85 ± 0.1	0.35 ± 0.05	0.35 ± 0.05	0.65 ± 0.20
	Monsoon (N =35)	180.5 ± 10.9	8.68 ± 6.6	1:4	12.55 ± 2.6	3.37 ± 1.4	0.25 ± 0.03	0.20 ± 0.01	0.60 ± 0.15
	Post monsoon (N =35)	185.1 ± 17.7	11.80 ± 7.2	4:3	9.29 ± 1.1	3.13 ± 1.9	0.25 ± 0.05	0.40 ± 0.10	0.70 ± 0.30

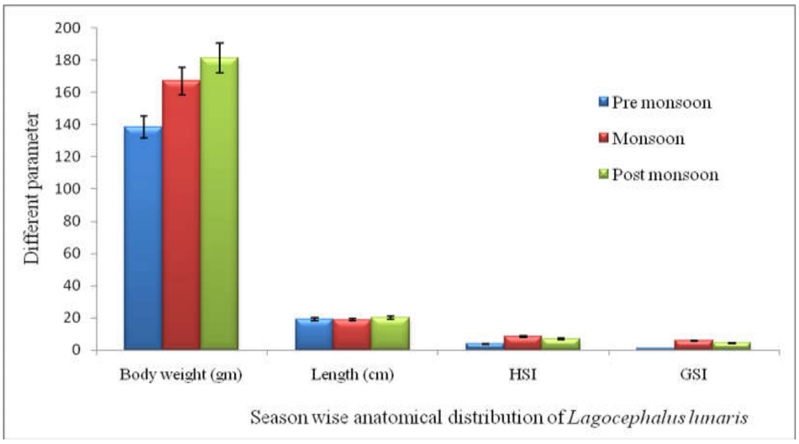

Figure 3. Seasonal Variation of body weight, length, HSI, GSI of *Lagocephalus lunaris* (Bloch and Schneider, 1801)

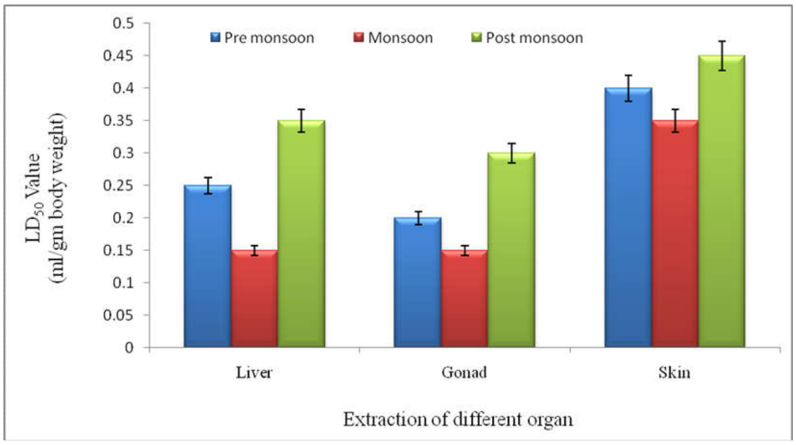

Figure 4. Toxicity test of *Lagocephalus lunaris* (Bloch and Schneider, 1801) LD_{50}

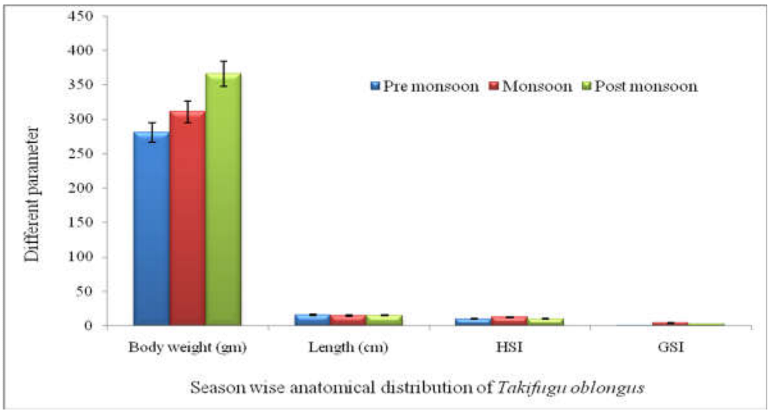

Figure 5. Seasonal variation of body weight, length, HSI, GSI of *Takifugu oblongus* (Bloch, 1786)

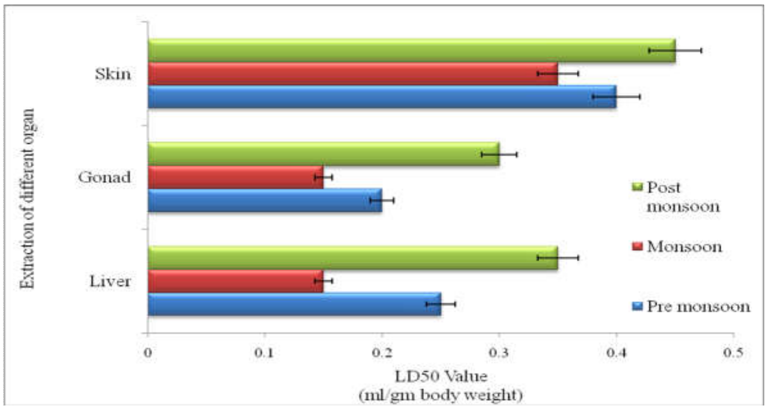

Figure 6. Toxicity test of *Takifugu oblongus* (Bloch, 1786) LD $_{50}$

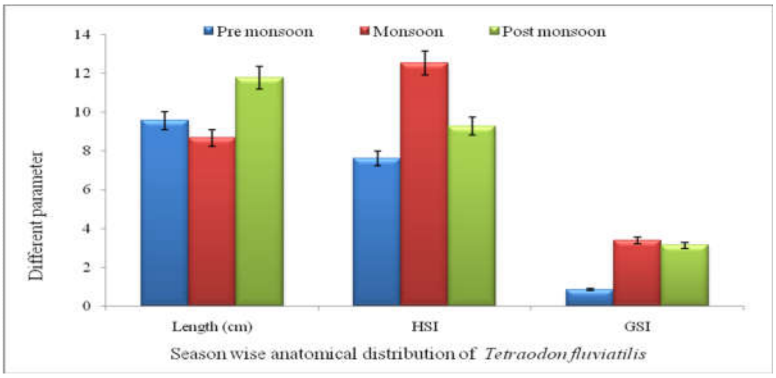

Figure 7. Seasonal Variation of body length, HSI, GSI of *Tetraodon fluviatilis* (Hamilton, 1822)

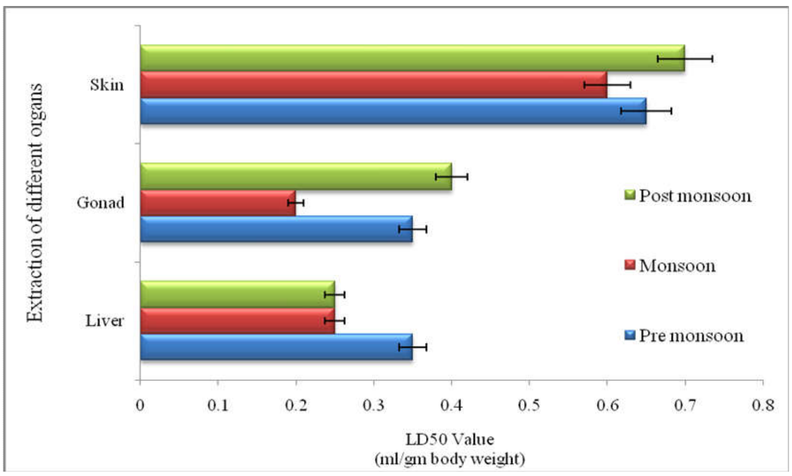

Figure 8. Toxicity test of *Tetraodon fluviatilis* (Hamilton, 1822) LD $_{50}$

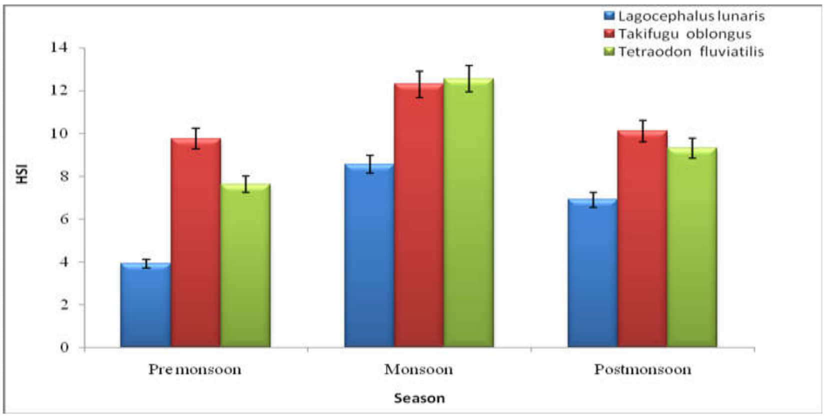

Figure 9. Comparative study of Hepato Somatic Index (HSI) of 3 Puffer fishes viz. *Lagocephalus lunaris* (Bloch and Schneider, 1801), *Takifugu oblongus* (Bloch, 1786), *Tetraodon fluviatilis* (Hamilton, 1822) in three seasons

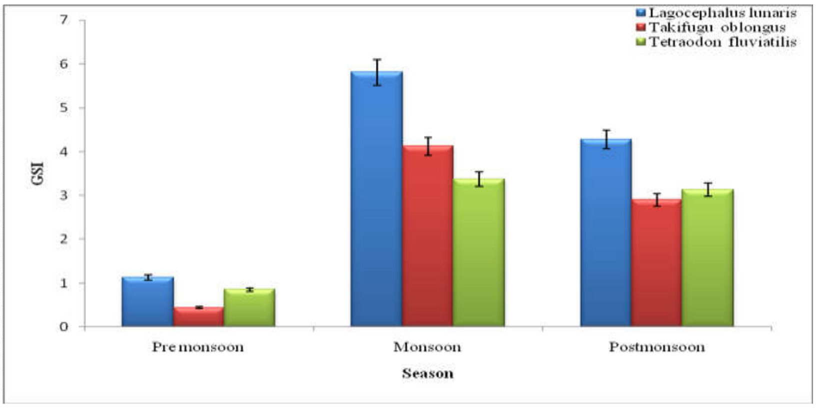

Figure 10. Comparative study of Gonado Somatic Index (GSI) of 3 Puffer fishes viz. *Lagocephalus lunaris* (Bloch and Schneider, 1801), *Takifugu oblongus* (Bloch, 1786), *Tetraodon fluviatilis* (Hamilton, 1822) in three seasons

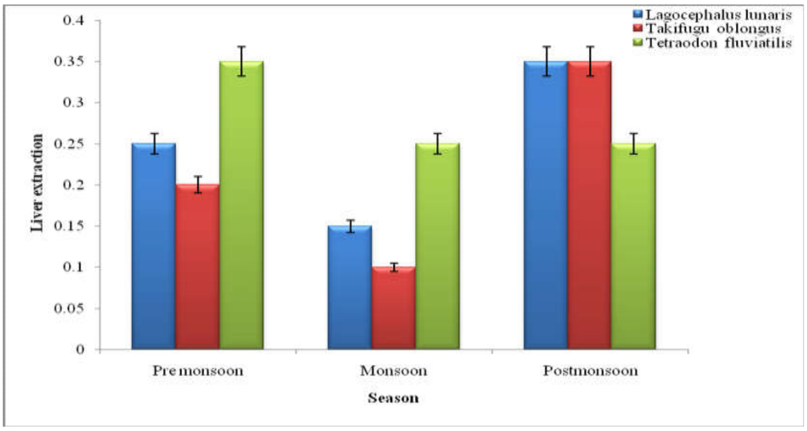

Figure 11. Comparative study of LD_{50} of Liver extract of 3 Puffer fishes viz. *Lagocephalus lunaris* (Bloch and Schneider, 1801), *Takifugu oblongus* (Bloch, 1786), *Tetraodon fluviatilis* (Hamilton, 1822) in three different seasons

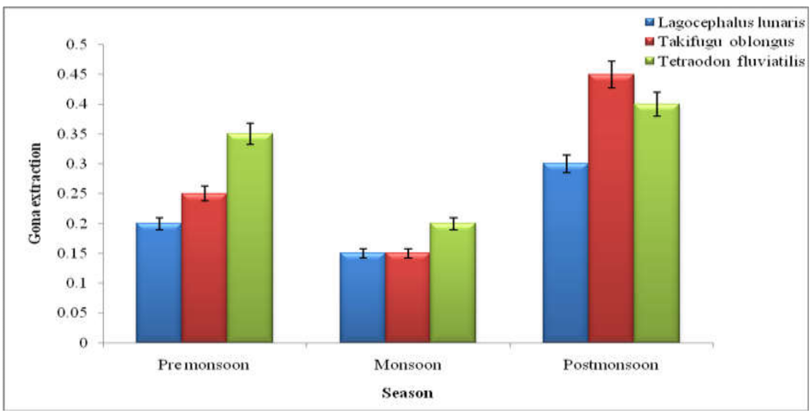

Figure 12. Comparative study of LD_{50} of Gonad extract of 3 Puffer fishes viz. *Lagocephalus lunaris* (Bloch and Schneider, 1801), *Takifugu oblongus* (Bloch, 1786), *Tetraodon fluviatilis* (Hamilton, 1822) in three different seasons

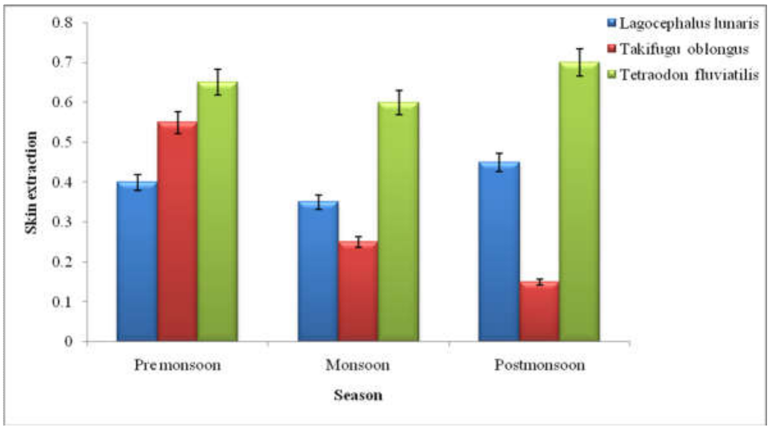

Figure 13. Comparative study of LD_{50} of Skin extract of 3 Puffer fishes viz. *Lagocephalus lunaris* (Bloch and Schneider, 1801), *Takifugu oblongus* (Bloch, 1786), *Tetraodon fluviatilis* (Hamilton, 1822) in three different seasons

Then, the fishes were injected with 0.1, 0.2 and 0.3 ml of liver extract/gm body weight in the experimental tanks (in triplicate). Similarly the three different concentrations of ovary and skin extracts were also injected (in triplicate) and were monitored continuously. In the second set of experiment, liver, ovary and skin extracts of puffer fishes were injected in increasing concentrations and mortality rate was observed and LD_{50} values were determined. LD stands for "Lethal Dose". LD_{50} is the quantity of a substance, given all at once, which causes the demise of 50% (one half) of a group of test animals. The LD_{50} is a technique to evaluate the short-term toxicity or acute toxicity of a toxic chemical. It is usually expressed as the amount of chemical administered (e.g., milligrams) per 100 grams (for smaller animals) or per kilogram (for bigger test subjects) of the body weight of the test animal. The LD50 can be found for any route of entry or administration but dermal (applied to the skin) and oral (given by mouth) administration methods are the most common.

RESULTS AND DISCUSSION

Accumulation of TTX is high in the liver, gonads, and skin of the puffer fish (Fuchi *et al*, 1991; Y. Mahmud *et al,* 2002; Panichpisal *et al,* 2003). Predominantly in marine species of puffer fish, liver and ovary showed the highest toxicity (more than 1000 MU/g), followed by skin (Mandal *et al,* 2013; Noguchi *et al,* 2006). Most of the experiments on puffer fish showed the liver to be the most toxic part of puffer fish and the muscle being the least toxic (Nagashima, 1999; Hashimoto, 1976; Matsui *et al,* 1981; Saoudi *et al*, 2008). On the contrary, this study shows that the ovaries can be more toxic than liver because toxin transfer to the skin decreases somewhat on the onset of spawning season and most of the TTX taken up into the liver would be transported to the ovary, presumably with the precursor of yolk proteins that are synthesized in the liver (Wallace, 1985; Specker and Sullivan, 1994).

Lagocephalus lunaris (Bloch and Schneider, 1801) is very abundant in almost all seasons in this coastal area. HSI and GSI are more in monsoon season which are 8.55 ± 1.5 and 5.81 ± 1.9 respectively (Table 3, Figure 9, 10). In *Lagocephalus lunaris* premonsoon LD_{50} value of liver extract, gonad extract and skin extract are 0.25 ± 0.05, 0.20 ± 0.01, 0.40 ± 0.07 ml/gm body weight respectively, in monsoon season LD_{50} value of liver extract, gonad extract and skin extract are 0.15 ± 0.01, 0.15 ± 0.07, 0.35 ± 0.09 ml/gm body weight respectively and in post-monsoon season LD_{50} value of liver extract, gonad extract and skin extract are 0.35 ± 0.09, 0.30 ± 0.05, 0.45 ± 0.04 ml/gm body weight respectively (Table 3). So, from the above result it is clearly observed that gonad is more toxic than liver and skin. LD_{50} value shows that in monsoon season toxicity of liver and gonad is almost same (Table 3, Figure 2A, 3, 4, 11, 12, and 13).

Takifugu oblongus (Bloch, 1786) is very plentiful in monsoon and post monsoon season and less abundant in pre monsoon season in this coastal area. HSI and GSI are more in monsoon season which are 12.29 ± 3.2 and 4.12 ± 1.3 respectively (Table 3, Figure 9, 10). In *Takifugu oblongus* premonsoon LD_{50} value of liver extract, gonad extract and skin extract are 0.20 ± 0.03, 0.25 ± 0.02, 0.55 ± 0.10 ml/gm body weight respectively, in monsoon season LD_{50} value of liver extract, gonad extract and skin extract are 0.15 ± 0.03, 0.10 ± 0.01, 0.25 ± 0.05 ml/gm body weight respectively and in post-monsoon season LD_{50} value of liver extract, gonad extract and skin extract are 0.35 ± 0.06, 0.45 ± 0.15, 0.15 ± 0.15 ml/gm body weight respectively (Table 3). So, from the above result it is clearly observed that gonad is more toxic than liver and skin. LD_{50} value shows that in monsoon season toxicity of liver and gonad is almost same (Table 3, Figure 2B, 5, 6, 11, 12, and 13).

Tetraodon fluviatilis (Hamilton, 1822) were plenty in monsoon and post monsoon season and less abundant in pre monsoon season in this coastal area. HSI and GSI are more in monsoon season which are 12.55 ± 2.6 and 3.37 ± 1.4 respectively (Table 3, Figure 9, 10). In *Tetraodon fluviatilis* premonsoon LD_{50} value of liver extract, gonad extract and skin extract are 0.35 ± 0.05, 0.35 ± 0.05, 0.65 ± 0.20 ml/gm body weight respectively, in monsoon season LD_{50} value of liver extract, gonad extract and skin extract are 0.25 ± 0.03, 0.20 ± 0.01, 0.60 ± 0.15 ml/gm body weight respectively and in post-monsoon season LD_{50} value of liver extract, gonad extract and skin extract are 0.25 ± 0.05, 0.40 ± 0.10, 0.70 ± 0.30 ml/gm body weight respectively (Table 3). So, from the above result it is clearly observed that gonad is more toxic than liver and skin. LD_{50} value shows that in monsoon season gonad is more toxic than of liver and skin (Table 3, Figure 2C, 7, 8, 11, 12, 13).

Conclusion

3 puffer fishes viz. *Lagocephalus lunaris* (Bloch and Schneider, 1801); *Takifugu oblongus* (Bloch, 1786); *Tetraodon fluviatilis* (Hamilton, 1822) in Digha coastal belt

of West Bengal, India, are studied for toxicological characterization. From the current study it is observed that in three puffer fishes toxicity level in monsoon season is highest as compared to other seasons. Gonad and liver tissue is more or less equally toxic in three puffer fishes. More lethal potency of gonad has a correlation with spawning and egg maturity.

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