

# Seasonal Toxicity Assessment of Oblong Blowfish (*Takifugu oblongus*) from Tamil Nadu Coast, India

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## ARTICLE INFO

### Article history:

Received on: 06/01/2017

Accepted on: 19/02/2017

Available online: 30/03/2017

### Key words:

Acute Toxicity, Food Intoxications, Histopathology, Mouse Bioassay, Pufferfish, *Takifugu oblongus*.

## ABSTRACT

Food intoxications by pufferfishes have been reported all over the world producing several deaths every year and toxicological profiling of various species of pufferfishes is crucial to avoid potential health hazards imposed by ingestion of them. The present study is focussed to assess the toxicity of the pufferfish *Takifugu oblongus*, from the coast of Tamil Nadu, India, in different seasons. The toxicity was evaluated by mouse bioassay using Swiss Albino mice which is expressed in mouse units (MU/g). Anatomical changes were observed which is followed by histopathology of the dead mice tissues to prove the toxicity. The toxicity of liver was at its maximum during the pre-monsoon season with 163 MU/g and skin showed least toxicity with 75.88 MU/g. Post-monsoon season results showed that gonads proved maximum toxicity with 249.06 MU/g, while skin showed lowest toxicity with 89.34 MU/g. Histopathology analysis of the dead mice showed various inflammations and degenerations in the brain, heart, liver and kidneys. The toxicity of the pufferfish followed a seasonal pattern differing in various seasons. The present study evidently proved that *T. oblongus* is highly toxic and consumption of the same can pose serious dangers of health hazards and possibility of lethality to humans.

## INTRODUCTION

Marine Ecosystem remains the habitat of organisms from various phyla amidst which fishes are the most diversified group. Several toxic marine organisms are reported till date and fishes have exhibited toxicity at extremities. The coastline of the Bay of Bengal and Arabian Sea continues to be a rich fishing ground in the South Asian region and India is one of the world's largest marine natural products' nations. The south east coast of India extends its coastal waters from the state of Tamil Nadu to Odisha. Among the wide varieties of fishes found in these waters, the pufferfishes form reasonable quantities. The pufferfishes belong to the family Tetraodontidae which includes 189 species in 28 genera (Veeruraj *et al.*, 2011). Pufferfishes produce a potent neurotoxin named Tetrodotoxin (TTX) which is a sodium channel blocker in cell membranes. Reports indicated that TTX is much similar to saxitoxin (STX), which causes paralytic shellfish poisoning (How *et al.*, 2003).

Consumption of these pufferfishes had produced food intoxications including numerous deaths worldwide viz, Mexico, USA, Hong Kong, Japan, Korea, Taiwan, Malaysia, Bangladesh and even India is not spared (Lange., 1990; Ghosh *et al.*, 1993; Yang *et al.*, 1996; Loke *et al.*, 1997; Yoshikawa *et al.*, 2000; Vazquez *et al.*, 2000). Pufferfish poisoning kills approximately 20-100 people annually, in Japan as reported by Yamashita *et al.* (2000) and Narahashi *et al.* (2001). Ingestion of the cooked roe of *Chelenodon patoca* produced numerous deaths and it was the first report of pufferfish poisoning in India reported by Jones in 1956. The toxicity of pufferfishes vary based on varied species, sex, season of collection, geographical location, and anatomical distribution as well (Matsui *et al.*, 1998; Few *et al.*, 1998; Yu *et al.*, 2002). In India, occurrence of pufferfish poisoning is sporadic, yet documenting toxicological profiles of the pufferfishes is still inadequate. Dating back, few species of pufferfishes were assessed for toxicity from the Bengal Coast of India. Research on pufferfishes from southern parts of India is lacking. *Takifugu oblongus* (Bloch, 1786), commonly called the Lattice Blassop or Oblong Blowfish is one among the available species of pufferfish along the Indian coast.

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Nevertheless, the toxicity of this species has been reported elsewhere, Lattice Blassop is considered a delicacy in most of the countries (Chew *et al.*, 1983; Mahmud *et al.*, 1999). *T. oblongus* was a disaster to Bangladesh in the years 1998 and 2002 causing several deaths due to its toxin (Mahmud *et al.*, 1999; Ahmed., 2006; Homaira *et al.*, 2010). This study was undertaken to analyse the toxicity of the pufferfish *Takifugu oblongus* collected from the coast line of Tamil Nadu using Swiss albino mice.

## MATERIALS AND METHODS

### Specimen Collection

Specimens were collected from Kasimedu fishing harbour, Chennai, Parangipettai coast in Chidambaram and Mandapam coast in Rameswaram in three seasons viz., Pre-monsoon, Monsoon and Post-monsoon. A total of 67 specimens were collected during all the seasons but monsoon season showed no availability of samples. The specimens were tightly packed, transferred in Icebox to the Medical Biotechnology Lab, VIT University and they were maintained in a deep freezer at -20 °C until use.

### Experimental Animals

Male Swiss albino mice weighing between 18-22 g were issued by and maintained in the Animal House, VIT University for experimentation. The study had strictly followed the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA). The experimental procedures followed, were approved by the Institutional Animal Ethical Committee (IAEC), VIT University (Registration No: 197/BC/06/CPCSEA). Chloroform was used as an anaesthetic agent for control mice before dissections and handling of mice was done ethically to reduce pain and suffering.

### Preparation of the toxic extracts

The specimens were dissected and the organs particularly Skin, Muscle, Liver and Gonads were excised from which the extract was prepared. 10 g of every organ is weighed and separately homogenized in tissue homogenizer with 50 ml of 0.1% Acetic acid in water. The tissue homogenates were boiled for 10 minutes in water bath at 50 °C. They were cooled and centrifuged at 3000 rpm for 10 minutes. The supernatants were collected and stored. The same procedure was followed thrice and the supernatants collected were combined, filtered and stored (Khora, 1991).

### Mouse Bioassay

Mouse bioassay is the standard procedure for basic toxicity assessments. 15 Male Swiss albino mice weighing between 18-22 g were used for standard Mouse Bioassay. The mice were separated into 5 groups, with each group having 3 mice. First group is maintained as Control, 2<sup>nd</sup> group is injected with Liver

extract, 3<sup>rd</sup> with Skin extract, 4<sup>th</sup> with Muscle extract and 5<sup>th</sup> with Gonads extract. The control group receives 1 ml of 0.1% acetic acid. 1 ml of the each extract (undiluted) was injected into groups of mice split accordingly. If the mice die before 4 minutes, the extracts were diluted with 5 mg/ml Phosphate buffered saline (PBS). The injected mice were observed for toxicity symptoms and death for few minutes (Hollingworth *et al.*, 1990). The toxicity of the extracts in Mouse Units (MU g<sup>-1</sup>) were calculated by the dose-death time relationship, where 1 MU is defined as the amount of toxin (approximately 0.22µg) required to kill a 20 g male mouse of ddY strain within 30 minutes of injection (Ghosh *et al.*, 2004).

### Histopathological Examination

Brain, heart, liver and kidneys of the mice which died followed by injection of the toxic extracts were excised to ascertain the toxicity of *T. oblongus*. They were fixed in 10% formalin for 24h and processed further (Lefkowitz, 1987). Tissue sections were prepared for each organ, followed by Hematoxylin and Eosin (H&E) staining which is observed under light microscope and photographed.

## RESULTS

### Mouse Bioassay

As a result of mouse bioassay, the toxicity of the pufferfish *Takifugu oblongus* was determined. Muscles of the pufferfish showed no toxicity symptoms in the injected mice. In the pre-monsoon season, the maximum toxicity of the liver was found to be 163 MU/g, gonads were 143.33 MU/g, and skin was 75.88 MU/g. Whereas, an increase in toxicity was noticed in the pufferfish during the post-monsoon season. Gonads showed maximum toxicity with 249.06 MU/g, followed by the liver with 201.9 MU/g and skin with 89.34 MU/g. The degree of toxicity was also analysed with the calculated mouse units (MU).

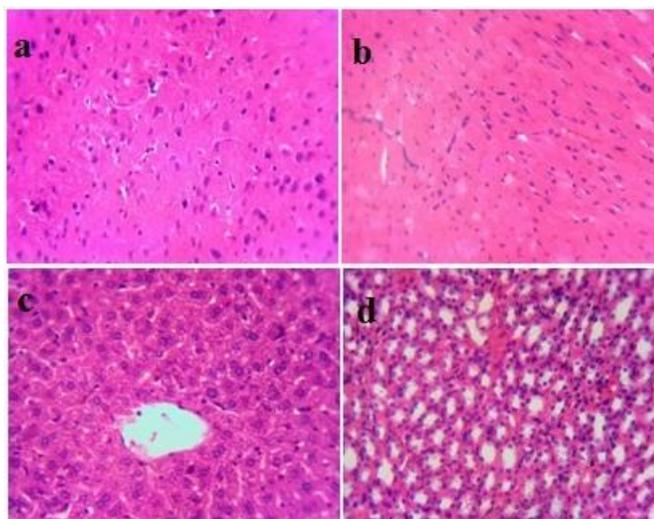
### Gross physiological and anatomical observations

The injected mice showed intense hyper activity by running inside the cage in an excited manner, escape reaction, shivering of the forelimbs, paralysis of the hind limbs, paleness of the tail, diarrhoea and were suffocating until its final gasping breath. Upon autopsy, blood clots were found in the anterior visceral parts. Dark discoloration was noticed in the heart and liver. These observations were further supported by the histopathological changes.

### Histopathology

#### Control

The control mice injected with 0.1% acetic acid showed no notable changes in either of the organs viz, brain, heart, liver and kidneys in both pre and post-monsoon seasons (Figures 1 a, b, d & d).



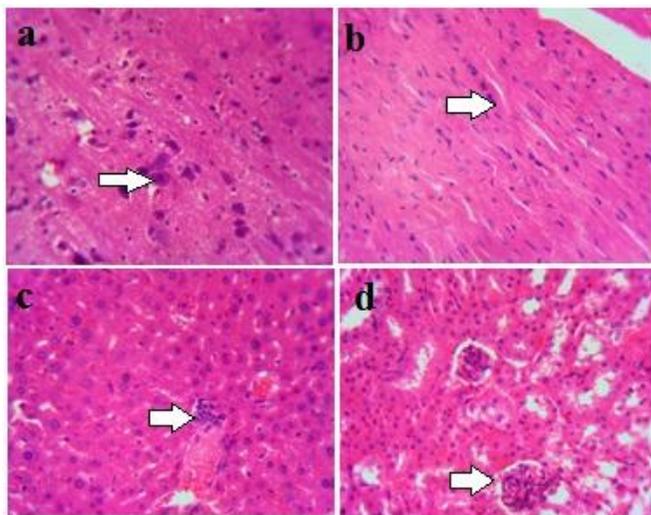
**Fig. 1:** Control Mice Images (H&E X400).

a. No prominent changes (Brain) 1b. No prominent changes (Heart) 1c. No prominent changes (Liver) 1d. No prominent changes (Kidneys)

### Pre-monsoon Observations

#### Effects of Liver Extract

Gliosis was observed in the brain of mice (Figure 2a). Heart tissues showed arrangement of cardiomyocytes in syncytial pattern and neither inflammations, nor infections were found (Figure 2b). Mild sinusoidal dilation and inflammations were found in the liver of mice (Figure 2c). Glomeruli with congestion and they are surrounded by closely packed tubules, lined by cuboidal epithelium were observed in the mice kidneys (Figure 2d).



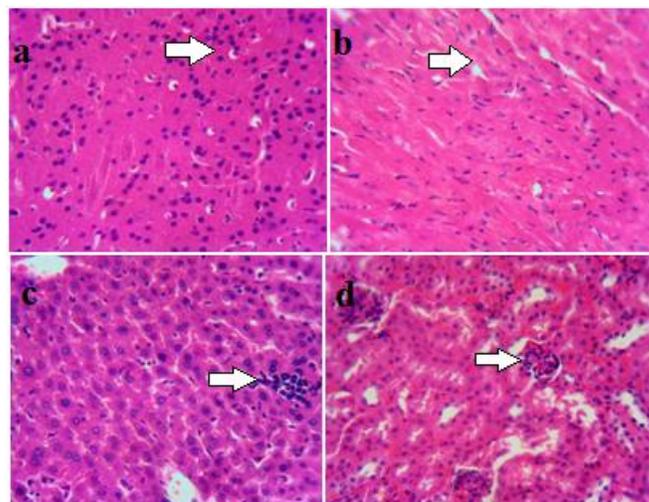
**Fig. 2:** Effects Of Skin Extract (Pre-Monsoon) On Mice (H&E X400)

a. Gliosis is seen (Brain) 2b. Cardiomyocytes seen in syncytial pattern (Heart) 2c. Mild sinusoidal dilations were seen (Liver) 2d. Glomeruli seen with congestion and closely packed tubules (Kidneys).

#### Effects of Gonads Extract

Gliosis with haphazardly arranged astrocytes was noticed in the mice brain (Figure 3a). Cardiomyocytes were arranged in syncytial pattern in the heart tissues of mice and neither

inflammations, nor infections were found (Figure 3b). Liver of mice showed mild to moderate periportal inflammation composed of lymphocytes, plasma cells and few eosinophils (Figure 3c). Glomeruli with congestion were seen in mice kidneys (Figure 3d).

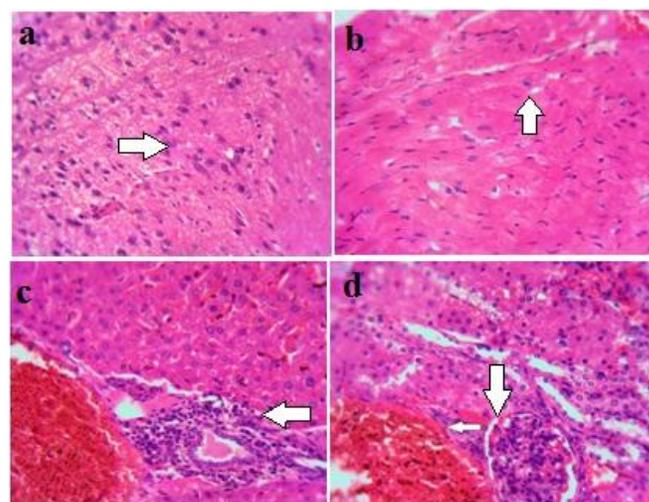


**Fig. 3:** Effects Of Liver Extract (Pre-Monsoon) On Mice (H&E X400).

a. Gliosis with haphazard astrocytes seen (Brain) 3b. Cardiomyocytes seen in syncytial pattern (Heart) 3c. Moderate periportal inflammation of lymphocytes seen 3d. Glomeruli with congestion seen (Kidneys)

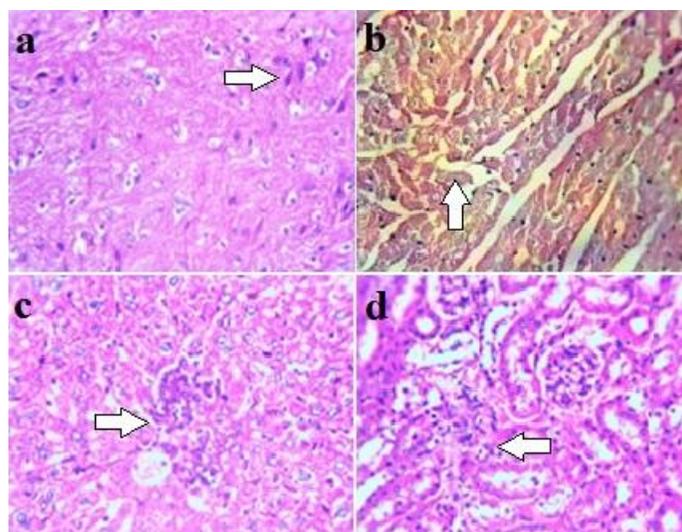
#### Effects of Skin Extract

Gliosis was seen and the molecular purkinje layer was observed with intact neurons in the brain of mice (Figure 4a). Cardiomyocytes were arranged in syncytial pattern in the heart tissues of mice and neither inflammations, nor infections were found (Figure 4b). Mild periportal inflammations were observed in the liver of mice (Figure 4c). Glomeruli with congestion were seen in mice kidneys (Figure 4d).



**Fig. 4:** Effects Of Gonads Extract (Pre-Monsoon) On Mice (H&E X400)

a. Gliosis noticed (Brain) 4b. Cardiomyocytes seen in syncytial pattern (Heart) 4c. Mild periportal inflammations seen (Liver) 4d. Glomeruli seen with congestion and closely packed tubules (Kidneys).

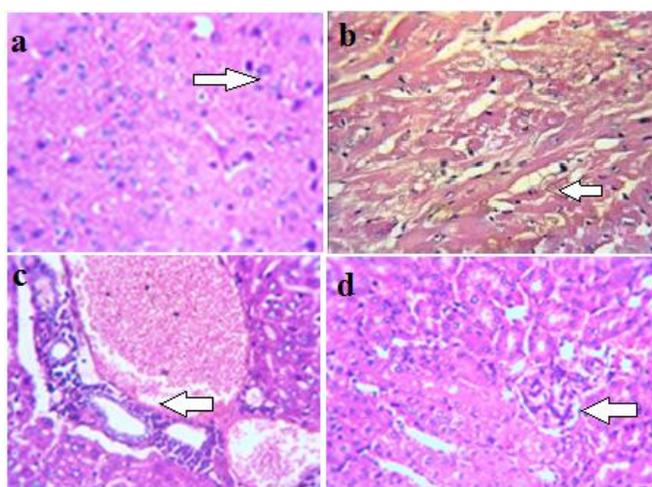


**Fig. 5:** Effects of Skin Extract (Post-Monsoon) On Mice (H&E X400). **a.** Mild vacuolar degenerations found (Brain) **5b.** Cardiomyocytes seen in syncytial pattern (Heart) **5c.** Periportal inflammation and mild congestions seen (Liver) **5d.** Glomeruli with hyper cellularity, mild congestions in the interstitia and medulla regions (Kidneys)

## Post-monsoon Observations

### Effects of Liver Extract

Mild vacuolar degenerations were seen in the cerebrum and cerebellum of mice (Figure 5a). Cardiomyocytes were arranged in syncytial pattern and no degenerative changes were observed in heart tissues of mice (Figure 5b). Periportal inflammation and mild congestion seen in mice liver (Figure 5c). Kidneys of mice showed glomeruli showed mild hyper cellularity in the cortex region, unremarkable vessels and tubules, mild congestion in the interstitia and mild congestion in the medulla regions (Figure 5d).



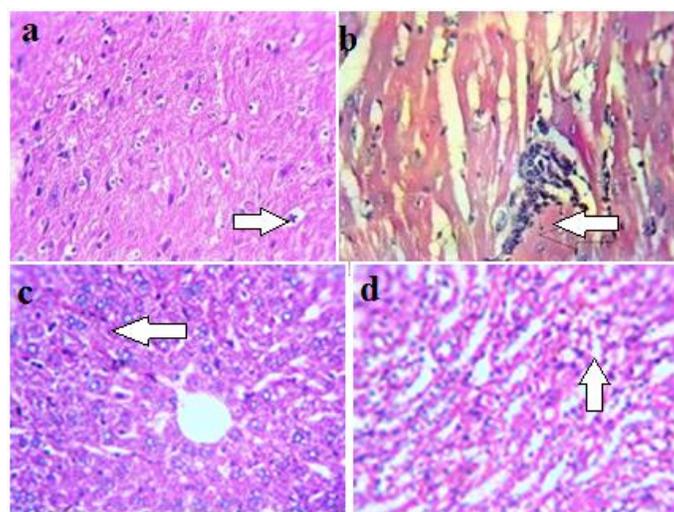
**Fig. 6:** Effects Of Liver Extract (Post-Monsoon) On Mice (H&E X400). **a.** Mild vacuolar degenerations seen (Brain) **6b.** Cardiomyocytes with mild degenerations in the cytoplasm seen (Heart) **6c.** Hepatocytic degenerations and mild congestions seen (Liver) **6d.** Unremarkable vessels and tubules with necrosis and apoptosis seen (Kidneys)

### Effects of Gonads Extract

Mild vacuolar degenerations were seen in the cerebrum and cerebellum of mice (Figure 6a). Cardiomyocytes with mild degenerative in the cytoplasm and no hypertrophy, fibrosis, necrosis, inflammation seen in mice heart (Figure 6b). Liver tissue showed degeneration of the hepatocytes, mild congestion and no apoptosis or necrosis (Figure 6c). In the cortex region of kidneys, glomeruli showed mild hyper cellularity, unremarkable vessels and tubules, mild congestion in the interstitia and mild congestion in the medulla regions were observed in the mice injected with liver and gonads extracts (Figure 6d).

### Effects of Skin Extract

No prominent changes were observed in the brain of mice (Figure 7a). Cardiomyocytes with focal vacuolar degeneration and myocardial focal inflammation were seen in heart tissue (Figure 7b). Periportal inflammation and hepatocytic degenerations were observed in mice liver (Figure 7c). Glomeruli showed mild hyper cellularity, mild congestion and tubular degeneration was seen in kidneys (Figure 7d).



**Fig. 7:** Effects Of Gonads Extract (Pre-Monsoon) On Mice (H&E X400). **a.** No prominent changes observed (Brain) **7b.** Cardiomyocytes with vacuolar degeneration and myocardial inflammations seen (Heart) **7c.** Periportal inflammation and hepatocytic degenerations found (Liver) **7d.** Glomeruli with hyper cellularity and tubular degenerations seen (Kidneys)

## DISCUSSION

Food intoxications by consumption of pufferfishes is a serious threat in almost all coastal countries. The principle objective of the study was to assess the comparative toxicity of the pufferfish *Takifugu oblongus* from the coast of Tamil Nadu in different seasons. Collection of specimens was done during three seasons viz, pre-monsoon (March-June), monsoon (July-October) and post-monsoon (November-December), in which there was no availability of the samples in the monsoon season. Pre-monsoon season showed more number of samples when compared to Post-monsoon season.

Males were predominant over the females. This report is quite juxtaposed with the study done by Ghosh *et al.*, in 2004, which reported that the frequent catch of *T. oblongus* along the Bengal coast was uniform throughout the year and females were predominant. Mouse bioassay revealed that the liver, gonads and skin of the pufferfish are highly lethal causing death of injected mice in few minutes. The muscle extract was completely non toxic showing no symptoms of pufferfish poisoning. In the pre-monsoon season, the toxicity of liver was maximum with 163 MU/g and skin showing least toxicity with 75.88 MU/g. Phenomenal changes were observed in the post-monsoon season, with gonads exhibiting extreme toxicity of 249.06 MU/g, which may be due to its maturation, and skin showing least toxicity of 89.34 MU/g.

Tani, in 1945 reported that toxicity  $\geq 10$  MU is considered weakly toxic (+),  $\geq 100$  MU is moderate (++) and  $\geq 1000$  MU is strongly toxic (+++), whereas,  $< 10$  MU is considered non-toxic (-). Kawabata in 1978 had reported that toxicity above 10 MU is hazardous for human consumption. This is in slight accordance with the study of Ghosh *et al* in 2004, which reported that ovaries were toxic than livers of the pufferfish *T. oblongus*, from the coastline of Bengal, in all the seasons.

The toxicity of *T. oblongus* in Taiwan waters was quite similar to that of this study indicating the most toxic organs were liver and ovaries and it varies with the seasons (Hwang *et al.*, 1988; Hwang *et al.*, 1992). Toxicity of the gonads would have increased possibly due to the increase in its weight, maturation and spawning season.

The present study is fairly in accordance with the toxicity of pufferfishes of Hong Kong waters, which follow a seasonal pattern (Yu *et al.*, 2002). In general, TTX is accumulated in the liver, gonads, skin and intestine, as reported by Fuchi *et al.*, in 1991 and Mahmud *et al.*, in 2000. Yet another study by Khora in 1991, reported that skin of the pufferfishes were most toxic. Lee *et al.*, in 2003 reported that TTX is accumulated in the pufferfishes through their food chain and indeed the toxicity varies depending on this factor too. Khora in 1994 reported that pufferfishes raised in captivity are non toxic. This study clearly shows the toxicity of *T. oblongus* is possessed in its liver and gonads at the maximum and it varies with the seasons.

## CONCLUSION

Conclusively, the pufferfish *T. oblongus* which has been the cause of food poisoning outbreaks and deaths in most countries proved its seasonal variation in toxicity in its liver and gonads at extremities. Hence, the consumption of this pufferfish species can be avoided to prevent the health hazards not only in the south eastern part of India but also in several other nations. The present study adds detailed data to the toxicity profiling of pufferfishes from India especially from southern India.

## ACKNOWLEDGEMENTS

The authors are grateful to the authorities of VIT University, Vellore, Tamil Nadu, India, for providing the required facilities, their constant encouragement and support.

## CONFLICTS OF INTEREST

The authors hereby declare that we have no conflicts of interest.

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#### How to cite this article:

Indumathi SM, Khora SS. Seasonal Toxicity Assessment of Oblong Blowfish (*Takifugu oblongus*) from Tamil Nadu Coast, India. *J App Pharm Sci*, 2017; 7 (03): 188-193.