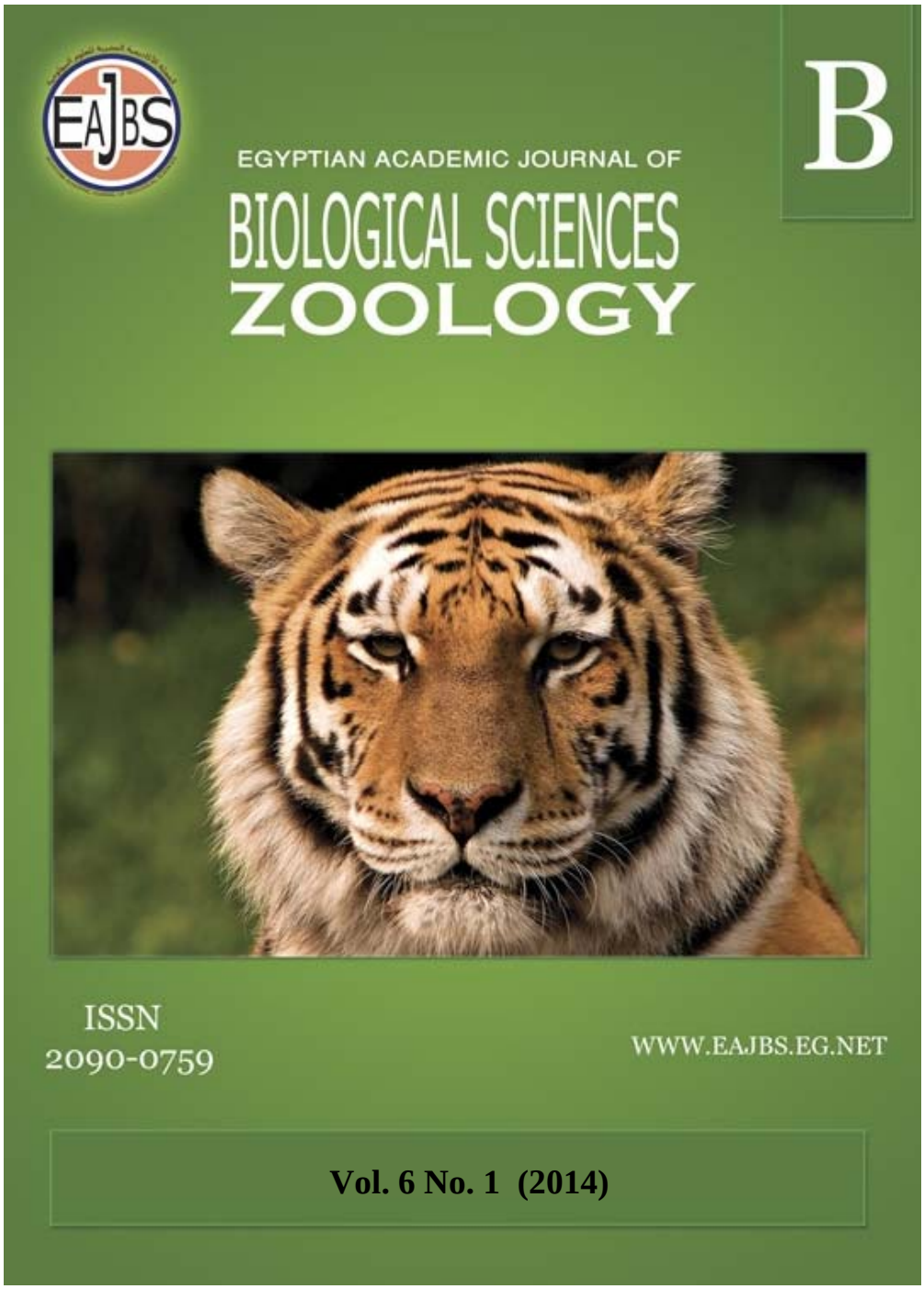

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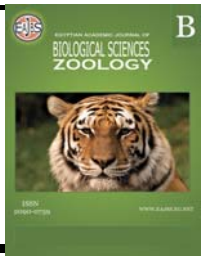

Vitamin-C work as an antidote against bisphenol-A toxicity in freshwater fish *Cirrhinus mrigala* (Ham.)

Sarita Murmu* and Vinoy K. Shrivastava
Dept. of Zoology, SSLNT Mahila College Dhanbad Vinoba Bhave University, Hazaribag Jharkhand (India)
sarita08_09@yahoo.com

ABSTRACT

Bisphenol-A (BPA) or 2,2-bis (4-hydroxyphenyl) propane is a compound widely used in the production of polycarbonate and other plastic industries. In this study, 45 fishes (*Cirrhinus mrigala)* weighing 90 ± 5 g were used and divided into 3 groups of fifteen each. 1st Group received fish diet only and served as control, 2nd group were exposed with Bisphenol-A (2mg/l) and 3rd group were exposed with vitamin-C (50 mg/l) along with Bisphenol-A (2mg/l), respectively for 15, 30 and 60 days and the protein contents were estimated in brain and testis. The significantly increased in protein levels were noticed in brain and testis after 15, 30 and 60 days of Bisphenol-A exposed as compared to control group. While, the animals supplemented with vitamin-C along with Bisphenol-A were showed insignificantly decreased in protein level in brain and testis after 15 and 30 days as compared to Bisphenol-A group. However, this levels were significantly lowered in brain and testis after 60 days as compared to Bisphenol-A. These results indicate that vitamin-C work as an antidote against Bisphenol-A toxicity in freshwater fish *Cirrhinus mrigala.*

Keywords: Bisphenol-A, Vitamin-C, Brain, Testis, Protein, *Cirrhinus mrigala.*

INTRODUCTION

Bisphenol-A (BPA) or 2,2 bis(4-hydroxyphenyl) propane is a compound used widely in the production of poly carbonate and other plastics and flame retardants (Alexander *et al.,* 1988). Final products include adhesives, coatings, paints, building materials, thermal papers, etc. (Staples *et al.*, 1998). BPA is a solid substance under ambient conditions, and it can be purchased as crystals, prills or flakes. BPA waste may enter the environment during handling, loading and unloading heating, as accidental spills or releases. Bisphenol-A is commonly detected in environment (Staples *et al.,* 2000) and in food products (Brotons *et al.,* 1995). BPA is an environmental disruptor and it has an estrogenic activity on nearly all classes of vertebrates (Maruyama *et al.,* 1999).

Gonad morphology of fish was affected at 1 and 10µg/L of BPA, respectively for female and male sticklebacks (De Kermoysan *et al.,* 2013). BPA has been

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associated with hormonal, morphological, functional, and behavioral anomalies related to reproduction (Adriani *et al.*, 2003), including alterations in the testosterone levels at birth in rats (Tanaka *et al.*, 2006), in brain development in female mice (Tando *et al*., 2007), in rat prostate development (Ramos *et al*., 2001), in the male and female genital tract (Maffini *et al.*, 2006), and in the tissue organization of the rat and mouse mammary gland (Munoz-de-Toro *et al*., 2005).

Ascorbic acid (Vitamin C) is essential for producing collagen and bone minerals, assists in metabolizing iron and helps in activation of Vitamin D. Ascorbic acid (Vitamin C) is as essential nutrient in aqua feeds and is an indispensable nutrient required to maintain the physiological processes of different animals including fishes (Tolbert, 1979) effects of hormones produced by the adrenal gland during prolonged periods of stress (Lovell, 1989; Navarre and Halver, 1989). Also, it has an important role in a great number of biochemical process such as synthesis of collagen which is an intercellular protein and principal constituent of skin, scales, mucosa, cartilaginous tissues, bones and conjunctiva tissue formation, which involves all the organs of the body (Mc Dowell, 1989). Agarwal *et al.,* (1978) reported that high levels of ascorbic acid are efficient to enhance tolerance to environmental stressors e.g. aldrin toxicity. In this study, vitamin-C work as an antioxidant against bisphenol-A toxicity and to see if vitamin C could reverse the effect of bisphenol-A induced in brain and testis of fish *Cirrhinus mrigala*.

MATERIALS AND METHOD

45 fishes *Cirrhinus mrigala* weighing 90±5 g were acclimatized in the laboratory condition prior to initiation of the experiment in the month of December to February. The fishes were divided into 3 groups of fifteen each. Ist group received fish diet only and serve as control while 2nd group were exposed with Bisphenol-A (2mg/l) and 3rd group were exposed with Vitamin-C (50mg/l) alongwith Bisphenol –A for 15, 30 and 60 days. All fishes were sacrificed and the brain and testis were dissected out quickly, weighed and homogenated in chilled 0.85% KCl solution for protein estimation and centrifuged for 15 minutes at 3000 RPM in a centrifuged machine. Supernatant were kept at cooling refrigerator and the protein level were estimated by adopting the appropriate methodology Protein by Lowry method (1951). The 'p' value was calculated by student ’t’ test. The comparison of the control data vs. treated was statistically analyzed by using ‘t’ test to established the validity of the investigation (Fisher and Yates, 1953).

RESULTS

Fishes (*Cirrhinus mrigala*) treated with Bisphenol-A (2mg/l) changed the colour of the fish skin (light brickish). Beside this, fish treated with Bisphenol-A (2 mg/l) supplemented along with vitamin C (50mg/l) shows recovery in their skin (silver black colour). It has also been observed that fishes exposed with Bisphenol-A (2mg/l) and Vitamin C (50mg/l) alongwith Bisphenol-A in brain and testis upto 15, 30 and 60 days showed alteration in different duration. Bisphenol-A significantly increased the protein level in brain and testis after 15, 30 and 60 days as compared to control group (Figs.1 and 2). Initially it shows lowering of values in brain and testis after giving vitamin-C along with Bisphenol-A exposures (Figs.1 and 2). Whereas, these values were insignificantly decreased in 30 days as compared to Bisphenol-A exposures

group (Figs.1 and 2). However, after 60 days it shows significantly decreased in brain and testis as compared to Bisphenol-A exposures group (Figs. 1 and 2).

Fig.1: Protein level mg/g concentration in brain of *Cirrhinus mrigala* after Bisphenol-A and vitamin-C supplemented along with Bisphenol-A exposures.

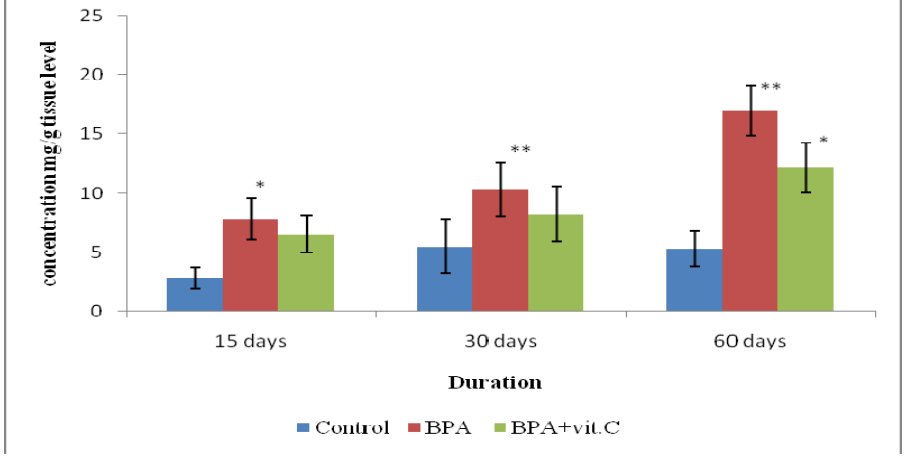
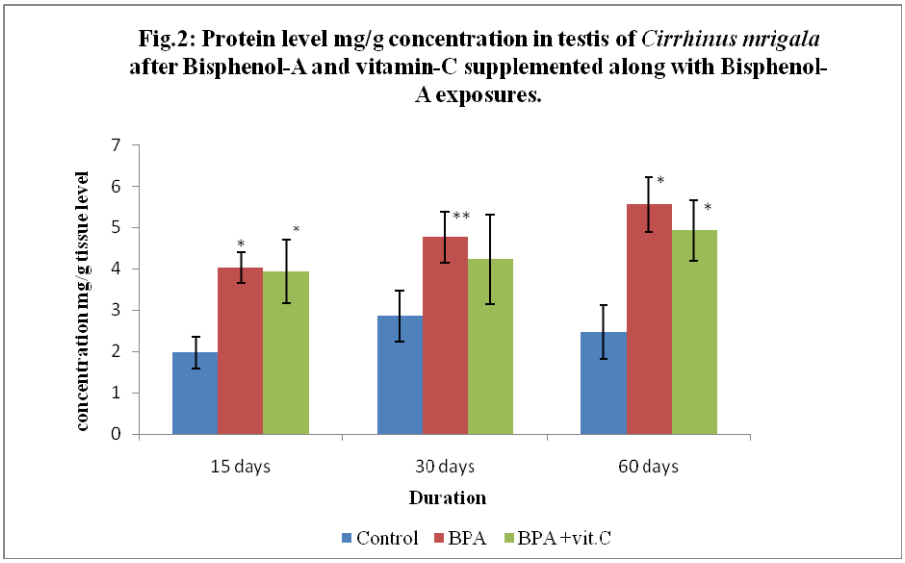


Fig.2: Protein level mg/g concentration in testis of *Cirrhinus mrigala* after Bisphenol-A and vitamin-C supplemented along with Bisphenol-A exposures.



Values are mean ± SEM of 5 fishes.
*Significantly values ($P < 0.05$) from control vs experiment by student 't' test.
**Significantly values ($P < 0.01$) from control vs experiment by student 't' test.

DISCUSSION

BPA doses cause reproductive toxicity and affect cellular development in rats and mice (Morrissey *et al.*, 1987). Experimental studies suggest that BPA adversely affects male reproductive system in general and testis in particular (Ohta *et al.*, 2011; Jain *et al.*, 2011). Tyler and colleagues (1999) found significant induction of the protein in fish exposed to estradiol from 24 hr after fertilization through 30 days after hatch. Larsen *et al.*, (2006) has observed that 67% of significantly altered proteins showed the same response (up or down regulated) in NP (Nonphenyl) and BPA exposed animals (males and females combined). The rest were either specific to NP (10%), BPA (19%) or they showed opposite responses to the two chemicals (4%). In contrast, only 20% of significantly altered proteins were common for NP and BPA exposed turbot: 60% were altered only in NP and 17% only in BPA. In present

investigation, it has been observed that bisphenol-A significantly increased the protein level in brain and testis after 15, 30 and 60 days as compared to control group.

The obtained results revealed that toxicated fishes were recovered when exposed with ascorbic acid (Vitamin C). However, fish growth is highly flexible and is one of the complex activities where it represents the net outcome of a series of environmental and physiological factors that begin with food intake, digestion, absorption, assimilation and other metabolic activities. All these processes may affect the final fish product (Bugaev *et al.,* 1994). Therefore, it could be concluded that fish exposed with ascorbic acid (Vitamin C) is efficient for reduction of Bisphenol-A toxicity. These results are much expected because ascorbic acid (Vitamin C) is closely related to the immunological system performance, and has antioxidant properties, favors integrity and fluidity of membranes (Brake, 1997) controlling the oxidizing reactions of fatty acids thus keeping cellular respiration and avoiding cell death (Verlhac and Gabandan, 1994). This antioxidant activity of ascorbic acid (Vitamin C) makes it a hunter of free radicals, thus preventing the autointoxication of immunological cells such as macrophages which are the first processors of the information about the alien bodies and maximizing the defense of fish (Brake, 1997). In fish the ascorbic acid concentrations were significantly observed in the testis (Dabrowski, 1991). In this study, it has been observed that protein level insignificantly lowered in tissues brain and testis after 15 and 30 days exposures as compared to Bisphenol-A group. While, protein level were significantly decreased in brain and testis after 60 days exposures as compared to Bisphenol-A group. These results indicate that vitamin-C work as an antioxidant against Bisphenol-A toxicity in fish *Cirrhinus mrigala.*

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