GLOBAL JOURNAL OF BIO-SCIENCE AND BIOTECHNOLOGY

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AMELIORATIVE ROLE OF VITAMIN-C AGAINST DIMETHOATETOXICITY IN AIR BREATHING FISH, *Clarias batrachus* (LINN.)

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ABSTRACT
The present investigation deals about the ameliorative role of vitamin-C against the toxicity of dimethoate on liver of *Clarias batrachus*. In this, total- 40 mature fishes were divided into four groups of ten each. Group Ist served as control and received normal diet *i.e.* Tokyo fish food (2g/fish), group IInd fed with normal diet and exposed with the dimethoate (45µg/l), group IIIrd received of dimethoate (45µg/l) and supplemented with vitamin-C (50mg/l),while, group IVth received vitamin-C (50mg/l) only for 30 and 60 days and hepatic enzymes activities *i.e.* GOT (Glutamate Oxaloacetate Transaminase), GPT (Glutamate Pyruvate Transaminase**),** ACP **(**Acid phosphatase), ALP (Alkaline phosphatase), hepatic protein content along with histomorphological changes were observed. Dimethoate increased the hepatic GOT, GPT, ACP, and ALP enzymes activities after 30 and 60 days in comparison to control group. While, the hepatic protein levels were lowered by dimethoate. Apart from this, dimethoate also revealed histomorphological degenerative changes characterized by congestion in blood vessels and cytoplasmic vacuolization with pycknotic nuclei in hepatic cells. However, the animal's supplemented vitamin-C along with dimethoate showed recoveries towards normalcy in their hepatic enzymes activities, protein levels and histomorphological changes after 30 and 60 days in comparison to dimethoate induced group. These results suggested that the toxicological effect of dimethoate alters morphological and physiological functions in hepatic tissues of *Clarias batrachus* by modulating the enzymological and biochemical changes. While, these changes can be ameliorated by vitamin-C because of its antioxidant properties.

KEY WORDS: Dimethoate, Enzyme activities, Histopathological, Vitamin-C, Liver, *Clariasbatrachus.*

INTRODUCTION:

Dimethoate (O, O-dimethyl S-methyl carbamoyl methyl phosphorodithioate), belong to organophosphate (OP) insecticides and it's a one of the major and most extensively used pesticides in agriculture along with household insects (Luis, 1997). It induces a number of morphological and physiological changes in several tissues and organs of the body and displays the ability to induce free-radical processes (Sharma *et al*., 2005). Dimethoate poisoning is usually block neuromuscular transmission in both animals and humans (De-Bleecker *et al.,* 1993). The signs of the toxicity of dimethoate in fish, *Channa punctatus* included jumping, erratic movement, imbalance and death (Dikshit and Raizada 1981). Several workers have been also reported that the organophosphate induced histopathological changes in the kidney of freshwater fish, *Puntius conchonius* and *Channa punctatus* (Banerjee and Bhattacharya 1994). Apart from this, it has been also reported that dimethoate inhibits cholinesterase enzyme activities in fresh water fishes (Perretet *et al.,* 1996). As we know that vitamin-C (ascorbic acid) is a water-soluble antioxidant and its role is to neutralize free radicals by donating electrons to free radicals such as hydroxyl and superoxide radicals (Bendich 1990). Vitamin-C protects the DNA of the cells from the damage caused by free radicals and mutagens. It prevents harmful genetic alteration with in cells and protects lymphocytes from mutations to the chromosomes (Gaby and Singh 1991). It also prevents free radical damage in the lungs and may even help to protect the central nervous system from such damage (Kronhausen *et al*., 1989). Moreover, vitamin-C also protects us by preventing the development of nitrosamines, the cancer-causing chemicals that stem from the nitrates contained in many foods (Gaby and Singh, 1991). In aquatic system various reports showed that vitamin-C acts as an antioxidant for various aquatic species and protect from poisoning of various hazardous chemicals including heavy metals, synthetic chemicals, pesticides and domestic wastes (Ambali *et al.,* 2007). So looking to it, in present investigation we tried to evaluate the protective role of vitamin-C against dimethoate toxicity by observing enzymological and histopathological changes in liver of air breathing fish *i.e. Clarias batrachus* after different durations *i.e.* 30 and 60 days.

MATERIALS & METHOD

40-sexually mature male catfishes (*Clarias batrachus*) weighing 150±10 grams were brought from the market, kept into the aquarium (size 18x30 inches) with sufficient water, maintained (by changing water alternate day) and acclimated in the Laboratory of Endocrinology, Department of Bioscience, Barkatullah University, Bhopal (M.P.), India for 15 days prior to initiation of the experiment. All fishes were divided into four groups of

ten each. Group I[st] served as control and received normal diet *i.e.* Tokyo fish food (2g/fish), group II[nd] fed with normal diet and exposed with the dimethoate (45µg/l), group III[rd] received of dimethoate (45µg/l) and supplemented with vitamin-C (50mg/l),while, group IV[th] received vitamin-C (50mg/l) only for 30 and 60 days. The fishes were sacrificed on 31[st] and 61[st] day intervals and their livers were dissected out quickly, washed, dried, weighed and some portion were fixed in Bouin's fixative for histomorphological studies, and rest portion were used for enzymological and biochemical parameters. For histomorphological studies, 5 µ thick paraffin sections were cut and stained with haematoxylene and eosine (H&E) adopting the methodology of Ehrlich, (1986). While, the total protein and enzymological estimation *i.e.* ACP, ALP and GOT, GPT were done by the adopting the methodologies of Lowry *et al.* (1951), Bergmeyer (1963) and Reitman & Frankel (1957) respectively.

RESULTS

In histomorphological observation, the hepatic cells of control and vitamin-C only treated group showed normal histoarchitectural structure characterized by polygonal and hexagonal hepatocytes have large amount of cytoplasmic materials with prominent nuclei surrounded by bile canaliculi (Fig.1 & 2). The liver of 30 days treated with dimethoate revealed histopathological degenerative changes characterization of congestion in blood vessels, leucocytic infiltration and cytoplasmic vacuolation with pycknotic nuclei (Fig.3). These changes were more prominent and severe in later part of the experiment *i.e.* 60 days of dimethoate treated group (Fig.4).

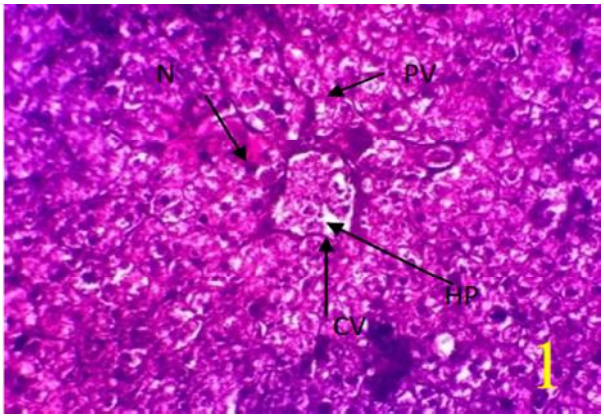


FIGURE 1: Showing the normal hepatic cell of control Liver *Clarias batrachus* (H&E X 400)

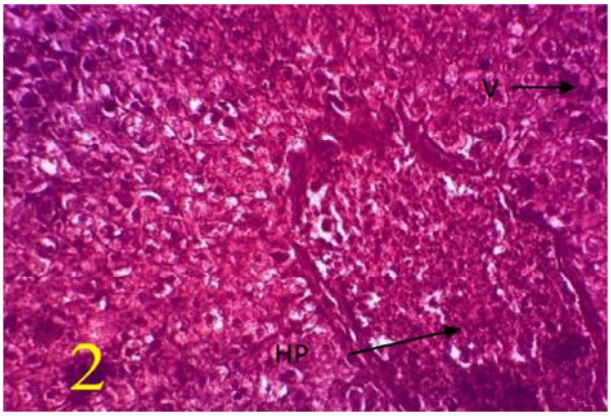
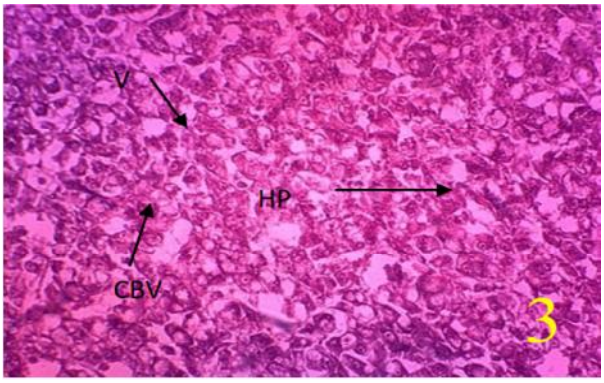


FIGURE 2: Section of Liver of fish, *Clarias batrachus* exposed to vitamin-C(50mg/l) for 30 and 60 days showing Hepatocyte (HP), Vein (V) (H & E. X 400).



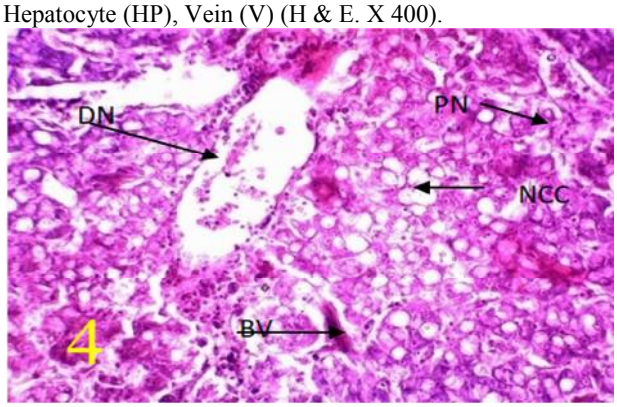


FIGURE 3 & 4: Section of Liver of fish, *Clarias batrachus* exposed to dimethoate(45µg/l) for 30 and 60 days showing damage of hepatocytes cells(HP), and vacuolization(V), pycknotic nuclei(PN),congestion in blood vessels(CBV), degeneration in cytoplasm(DN) (H&E X 400).

While, the animals supplemented with vitamin-C along with dimethoate showed some recoveries in their hepatic histoarchitectural structure characterized by defined hepatic cells and blood vessels in their hepatic histoarchitecture after 30 days and 60 days. The recoveries effects were more prominent in later part of the experiment (Fig. 5 & 6). In connection to this, the hepatic GOT, GPT, ACP and ALP enzyme contents were elevated by dimethoate in comparison to the control group (Fig. 7, 8, 9 & 10). However, a significant decreased in hepatic protein levels were noticed after 30 and 60 days dimethoate treated groups (Fig.11). But, the fishes supplemented with vitamin-C along with dimethoate lowered hepatic GOT, GPT, ACP and ALP levels towards normalcy in 30 and 60 days as compared to dimethoate treated groups.

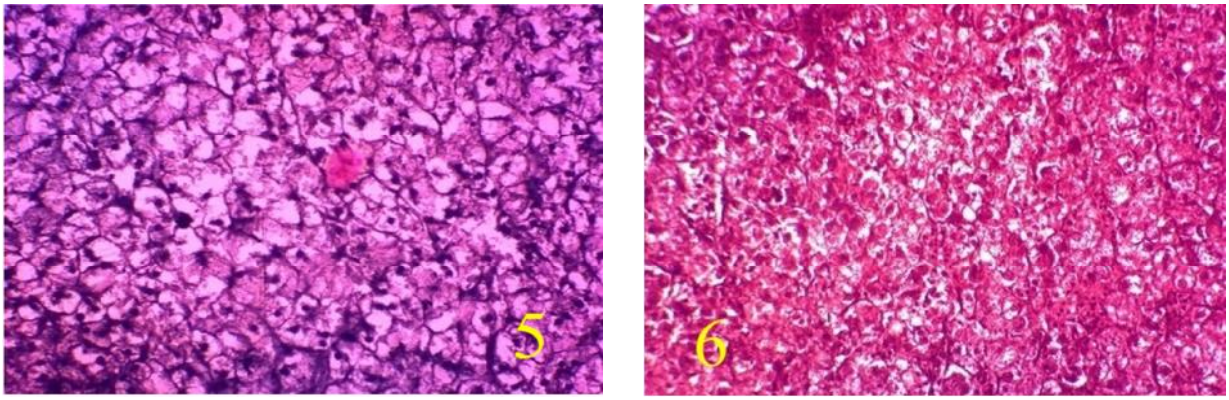


FIGURE 5 & 6: Showing the recoveries hepatic cell of Liver of *Clarias batrachus* exposed to dimethoate+ vitamin-C (45µg/l+50mg/l) for 30 and 60 days treated (H&E X 400).

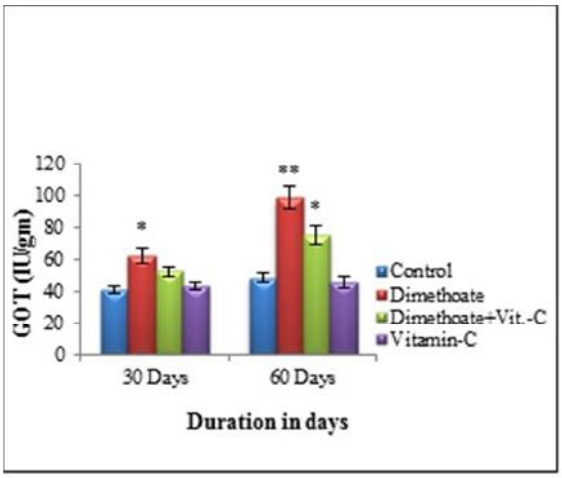


FIGURE 7: Hepatic Glutamate Oxaloacetate Transaminase (GOT) activities after different intervals *i. e.* 30 and 60 days treatment of dimethoate and dimethoate + vitamin-C supplemented, vitamin-C alone and control liver of fresh water fish *Clarias batrachus*.

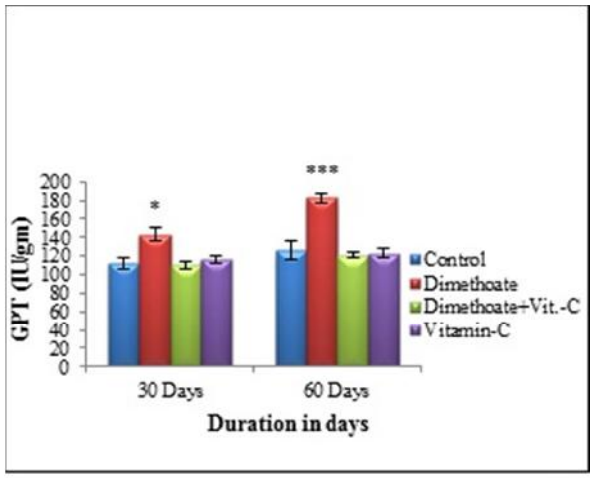


FIGURE 8: Glutamate Pyrophosphate Transaminase (GPT) activities after different intervals *i. e*. 30 and 60 days treatment of dimethoate and dimethoate treatment + vitamin-C supplemented, vitamin-C alone and control liver of fresh water fish *Clarias batrach*

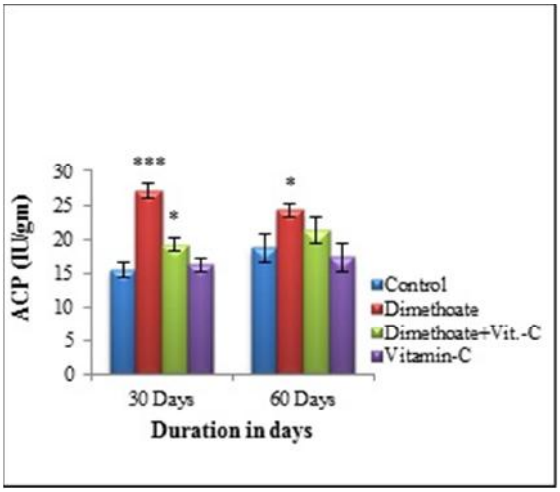


FIGURE 9: Acid phosphatase (ACP) activities (IU/gm) after different intervals *i. e.* 30 and 60 days treatment of dimethoate, dimethoate treatment + vitamin-C supplemented vitamin-C alone and control liver of fresh water fish *Clarias batrachus*.

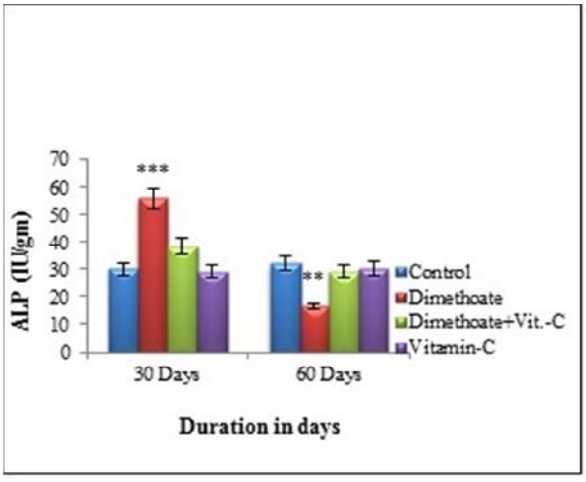


FIGURE 10: Alkaline phosphatase (ALP) activities (IU/gm) after different intervals *i.e*. 30 and 60 days treatment of dimethoate, dimethoate treatment + vitamin-C supplemented, vitamin-C alone and control liver of fresh water fish *Clarias batrachus*.

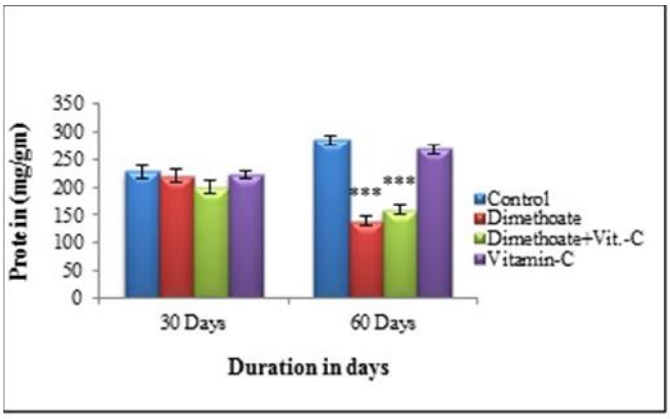


FIGURE 11: Protein estimation (mg/gm) after different intervals *i.e.* 30 and 60 days treatment of dimethoate and dimethoate treatment + vitamin-C supplemented vitamin-C alone and control liver of fresh water fish *Clarias batrachus*.

DISCUSSION

Most of the pesticides find their way into rivers, lakes and pond through flowing water and have been found to be highly toxic not only to fishes but also to the organisms which contribute to the food chain comprising of fishes (Anees, 1975). Dimethoate is organophosphates of the most widely used pesticide throughout the word in agriculture, home kitchen, gardens and veterinary practices. It is highly soluble in water and it adsorbs only very weakly to soil particles. As we know that the pesticides produces various types of deformities changes in aquatic and terrestrial animals. Gaafar *et al*. (2010) showed that exposure of organophosphate edifenphos pesticide to an *Oreochromis nilotichus*, the hepatic tissue showed congestion with various degrees of degenerative changes starting firstly with granular degeneration then vacuolar degeneration with progression towards hepatic cell necrosis after one week of exposure. These changes may be attributed to direct toxic effect of edifenphos on hepatocytes since the hepatopancres is the site of detoxification of all types of toxins and chemicals (Robert, 2001). In connection to this, it has been also reported that organophosphate fenvalerate induced changes in the normal architecture of liver (hepatocyte swelling and pyknotic nuclei) and muscles of *Clarias gariepinus*(Peebua *et al.*, 2008). Apart from this, it has been well knows that Vitamin-C has been reported to have a protective effect on rat liver injury (Shiraishi *et al*., 1993) and liver Carcinogenesis (Iverson *et al*., 1988) induced by cadmium and aflatoxin respectively. Our study also reported that the dimethoate induced histopathological and biochemical changes in liver of *Clarias batrachus* after different intervals. Dimethoate revealed histopathological degenerative changes characterized by congestion in blood vessels, leucocytic infiltration and cytoplasmic vacuolization with pycknotic nuclei, lowered protein contents and elevated enzyme activities especially in hepatic GOT, GPT, ACP and ALP levels. While, these changes were more prominent and severe in later part of the experiment. However, the animals supplemented with vitamin-C along with dimethoate showed some recoveries or towards normalcy in their hepatic histoarchitectural structure, protein content and enzymes levels after 30 days and 60 days. The recoveries effects were more prominent in later part of the experiment. The increase in the hepatic alkaline phosphate activity was due to the cellular damages caused by hepatotoxins or a response to overcome toxicity of organophosphate. Increase in GOT values indicate hepatocellular destruction and establish the hepatic cell breakage from their normal configurations. Abdul *et al*. (2004) and Venkateshwarlu *et al*. (1990) also reported that the pesticides caused significant increase in cellular damage which caused enhanced activity of phosphatases activity. So in our study, increased in GOT, GPT, ACP and ALP are the symptoms of induced injury along with hepatocellular necrosis and these changes were ameliorated by vitamin-C. In connection to this, the organophosphate significantly altered the level of total protein, free amino acid, nucleic acid, glycogen, lactate, enzyme protease, phosphatase alanine aminotransfarase, aspirate aminotransfarase and acetyl cholinesterase activity in liver and muscle tissues of *Channa punctatus* (Durga and Veeraiah, 2002; Tilak *et al*., 2005; Ganeshwade *et al*., 2012; and Nagaraju *et al*., 2013). Our observation also denote the lowering of protein in experimental fish under dimethoate may suggest that the dimethoate induced hepatic insufficiency and probably malnutrition or may be due to several pathological processes including decrease in liver protein synthesis and even protein elimination in the urine. Proteins are the building blocks, which is an essential constituent of food of animals. The data obtained in the present study has revealed that the levels of protein in the liver were significantly decreased after treated with dimethoate. Significantly decrease in protein might be due to catabolism of proteins and or malfunction of liver. The toxic stress may decrease protein content in tissues which is supported by Singh and Khare, (1999).

So, we may conclude that the toxicological effect of dimethoate alters morphological and physiological functions in hepatic tissues of *Clarias batrachus* by modulating the enzymological and biochemical changes. While, these changes can be ameliorated by vitamin-C because of its antioxidant properties.

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