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Antagonistic role of vitamin-C against dimethoate toxicity on kidney in air breathing fish *Clarias batrachus* (Linn.)

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KEYWORDS

Dimethoate, Histopathology, Vitamin-C, Kidney, *Clarias batrachus*

A B S T R A C T

The aim of present study was to observe the toxicity of organophosphate insecticide, dimethoate on kidney of *Clarias batrachus* and neutralizing role of vitamin-C against dimethoate induced toxicity. In this study, total 40 fishes were divided into four groups. Group I served as control, while Group II exposed with dimethoate ($45\mu gL^{-1}$), Group III received of dimethoate ($45\mu gL^{-1}$) and supplemented with vitamin-C ($50mgL^{-1}$) and Group IV received of vitamin-C ($50mgL^{-1}$) only for 30 and 60 days and enzymes activities *i.e.* GOT (Glutamate Oxaloacetate Transaminase), GPT (Glutamate Pyruvate Transaminase), ACP (Acid phosphatase), ALP (Alkaline phosphatase) and renal protein content along with histomorphological changes were quantified. In our study we find that the dimethoate caused a significantly increased renal GOT, GPT, ACP, ALP and protein level was decreased more prominently in the later part of the experiment as compared to control groups. The histological findings that the treated sections of the kidney showed glomeruli were shrunk and atrophied with degeneration. These changes were more severe in later part of the experiment *i.e.* 60 days dimethoate treatment, characterized by abnormal configurations with necrotic cells, some cells appeared with damaged proximal and distal tubules. The animals supplemented vitamin-C with dimethoate showed significantly recoveries in the renal cells of all experimental groups.

Introduction

Dimethoate is an organophosphate insecticide, widely used in developing countries for the protection of cultures and food-stuffs. It is readily absorbed through the skin (Secaucus, 1991). It is used against a wide range of insects, including aphids,

thrips, plant hoppers and whiteflies on ornamental plants, alfalfa, apples, corn, cotton, grapefruit, grapes, lemons, melons, oranges, pears, pecans, safflower, sorghum, soybeans, tangerines, tobacco, tomatoes, watermelons, wheat and other vegetables. It is also used as a residual wall spray in farm buildings for house flies. It has been administered to livestock for control of botflies. Dimethoate is available in aerosol spray, dust, emulsifiable concentrate, and ULV concentrate working of the nervous systems of both humans and insects. Dimethoate is of particular concern to those exposed formulations. The pollution of the environment plays a crucial role in the occurrence of many diseases affecting plants, animals and human. Organophosphorus insecticides are main factors of pollution in the environment Al-Haj *et al.* (2005). It is important to examine the toxic effect of organophosphate on fish, as they constitute an important link in food chain and their contamination by pesticides imbalances the aquatic ecosystem. The mechanism of the toxic action of dimethoate is one of inhibiting the acetyl cholinesterase and butyryl cholinesterase (BChE) activities (Jokanovic, 2001). Cholinesterase is an enzyme which is essential for the proper working of the nervous systems in humans.

A part from this, vitamin-C (ascorbic acid) is one of the many antioxidants and antioxidant is a substance, which slow down the damage that can be caused to other substances by the effects of oxygen and role is to neutralize free radicals by donating electrons to free radicals (Bendich, 1990). It is also called an antidote because, by donating its electrons, it prevents other compounds from getting oxidized (Nordberg and Arner, 2001). It is required for the growth and repair of tissues in all parts of our body. Ascorbic acid is an important co-factor involved in many biochemical functions and acts as a reducing agent (Elias and Oputiri, 2013).

So, In our the present this study we tried to evaluate the protective role of vitamin-C against dimethoate toxicity by observing enzymological and histopathological changes in kidney of air breathing fish *i.e. Clarias batrachus* after different durations *i.e.* 30 and 60 days.

Materials and Methods

Total 40-sexually mature catfishes (*Clarias batrachus*) weighing 150±10 g were brought from the market and acclimatized in the Laboratory condition, Department of Bioscience, Barkatullah University, Bhopal (M.P.) India for 15 days prior to initiation of the experiment. The fishes were kept in large aquariums (size 18x30 inches) which contained 25 liters of water, temperature 25±27oC, and change the water in alternate day. Dimethoate (30% EC) trade name Ah-Rogor-30 EC and vitamin-C (L-Ascorbic acid) obtained from V.K Traders M.P. Nagar, Bhopal, (M.P.) manufactured by Hindustan chemical and pesticide Kerala, Mumbai, (W.) have been used for the experiment. The fishes were divided into four groups of ten each. Group I served as control group received fish diet only, while Group II, exposed with dimethoate ($45\mu gL^{-1}$), Group III exposed with dimethoate ($45\mu gL^{-1}$) and supplemented with vitamin-C ($50mgL^{-1}$) dissolved in water for 30 and 60 days while Group IV supplemented with vitamin-C ($50mgL^{-1}$) only dissolved in water and fed with normal diet for 30 and 60 days. After the completion of different intervals *i.e.* 30 and 60 days fishes were sacrificed and their kidney was dissected quickly weighed and homogenized in 0.25M sucrose solution for enzymological studies *i.e.* Glutamate Oxaloacetate Transaminase, Glutamate Pyruvate Transaminase, Acid

phosphatase and Alkaline phosphatase levels were done by the adopting the methodologies of Reitman and Frankel (1957); Bergmeyer *et al.* (1963) respectively while, total protein were measured in kidney using the method of Lowry *et al.* (1951). For histopathological observations of kidney were dissected out, weighed and fixed in Bouin's fixative and after 24 hrs tissue processed for dehydration and prepare blocks in the wax and then 5 µm thick sections were cut by microtome and stained with Haematoxylene and Eosine (H&E) adopting the methodology of (Ehrlich, 1886). The significant values were calculated by using Student "t" test.

Results and Discussion

Normal histological structure of the kidney of control and vitamin-C alone supplemented animals showed well organized cellular structure, including well defined renal cells, proximal and distal convoluted tubules, bowman's capsule and uriniferous tubules (Figure 1) while, animals exposed with dimethoate for 30 days showed that renal vessels were dilated and congested, atrophic changes and vacuolization in uriniferous tubules. The nuclei of the lymphocytes are small or pycknotic nuclei (Figure 3). These changes were more severe in later part of the experiment *i.e.* 60 days dimethoate treatment, characterized by abnormal configurations with necrotic cells, vacuolization in uriniferous tubules, damage proximal and distal tubules and uriniferous tubules are tubules are disintegrating with pycknotic nuclei (Figure 5). But the animals supplementation of vitamin-C along with dimethoate are significantly ameliorate in 30 days treated and more significant recoveries were observed in 60 days treated groups as compared to dimethoate treated groups (Figures 4 and 6). In connection to this, the renal enzyme contents of ACP, ALP, GOT, and GPT were elevated by dimethoate in comparison to the control groups (Figures 7, 8, 9 and 10). However, a significant decreased in renal protein level were noticed after 30 and 60 days dimethoate treated group (Figure 11). But, the fishes supplemented with vitamin-C along with dimethoate lowered renal ACP, ALP, GOT and GPT levels in 30 and 60 days as compared to dimethoate treated groups (Figures 7, 8, 9 and 10).

The improvement of modern agriculture and crops production in large amount used of dimethoate is a cholinesterase inhibitor they acts as nerve agents. During the last decades, the extensive use of different pesticides in agriculture and for public health purposes, has led to drastic effects especially in animals and human (Pesticides residues in foods, 1996). It is also used for the indoor control of houseflies (Auta and Ogueji, 2006). Most of these chemicals are not highly selective but generally they provide to be toxic to many non-target species including man and other desirable forms of life that co-inhabit the environment, therefore, their improper application may result in serious illness and even death. Dimethoate, the insecticide used in this study, is a widely used organophosphate compound which has a significant contact and systemic action against a wide variety of insects and pests of both plants and animals (Westcott *et al.*, 1987). The fishes are the best indicator of water body pollution and kidney of fishes receives much the largest proportion of post bronchial blood and therefore renal lesions might be expected to be good indicators of environmental pollution (Juan *et al.*, 2003). Our study also reported that the dimethoate induced histopathological and biochemical changes in kidney of *Clarias batrachus* after different intervals. However, the animals

supplemented with vitamin-C along with dimethoate showed some recoveries or towards normalcy in their renal histoarchitectural structure, protein content and enzyme levels after 30 days and 60 days. Vitamin-C is an antioxidant and it is a chemical sub-stance that stops or controls the effects of a poison. Magar and Afsar (2013) also reported that necrosis, disintegrated cytoplasmic material and swelling of reneal tubules in *Channa punctatus* against organophosphate toxicity. Similar observations have been reported by (Aliaa *et al.,* 2011; Sanjoy and Rita, 2012) with various organophosphate insecticides. The histopathological changes in kidney lead to cloudy swelling in renal tubules, severe necrosis and cellular hypertrophy in *Cirrhinus mrigala* after exposure of organophosphate pesticide (Tilak *et al.,* 2005). The present observation gets support from the work of Gaafar *et al.* (2010), Aliaa *et al.* (2011) and Deka and Mahanta (2012). Many studies reported that organo-phosphorous compounds; dimethoate, may induce oxidative stress leading to the generation of free radicals and alterations in antioxidant and scavengers of oxygen-free radicals which alter structural and functional integrity of cell membrane (Bagchi *et al.,* 1995; Ahmed *et al.,* 2000; Glover *et al.,* 2007 and Boran *et al.,* 2012). The effect of dimethoate on the PCT revealed swelling of the mitochondrial membrane. Pragna *et al.* (2010) showed that the histopathological changes on kidney in *Oreocromis mossambicus* against dimethoate toxicity. It is also observed by Banaee *et al.* (2013) on Rainbow trout exposed to organophosphate. In connection to this, the enzymological and biochemical changes on renal tissue showed that the increased level of GOT, GPT, ACP and ALP while, the decreased level of protein due to the toxic effects of dimethoate for 30 and 60 days. Beside this, dimethoate also elevated significantly the levels of ACP, ALP in kidney after 30 and 60 days as compared to control group while, the fishes supplemented with vitamin-C along with dimethoate lowered renal GOT, GPT, ACP and ALP levels in 30 and 60 days as compared to dimethoate treated groups. Acid phosphate are hydrolytic lysosomal enzymes are released from the lysosomes for the hydrolysis of foreign material, hence it has a role in certain detoxification functions, it is also known as inducible enzyme activity in animal tissue goes up when there is a toxic impact and the enzyme being to counteract while, the ALP are the brush boder enzyme (Gold fisher *et al.,* 1964). Subsequently the enzyme activity may begin to drop either as a result of having partly or fully encountered the toxin or as a result of cell damage (Jaroli and Sharma, 2005). Higher value of ACP obtains from dimethoate toxicity in *Lepidocephalichthys thermalis* reported by (Sheela, 1992). The increased ACP values indicate renal cell breakage from their normal configurations. In our result also found that the decreased kidney protein level in experimental fishes, During the stress condition, fish needed more energy to detoxify the toxicants and to overcome stress or since fish have a very little amount of carbohydrates so next alternative source of energy is protein to meet the increased energy demand (Sambasiva, 1999). Similar results were reported by several workers Luskova *et al.* (2002), Pratibha *et al.* (2012) and Ganeshwade *et al.* (2012). Proteins are the building blocks, which is an essential constituent of food of animals. Patil (2011) reported that the decrease in the level of tissue protein may be due to excessive proteolysis to overcome the metabolic stress, as deposited protein in the cytoplasm can easily be used to replace the loss of proteins that occur during physiological stress. Almost similar finding reported by Remia *et al.* (2008) in fish *Tilapia mossambica* after

exposure to organophosphate insecticide like monocrotophos toxicity. During stress condition, fish needed more energy to detoxify the toxicants and to overcome stress. Tawfiki and Al-Badr (2012) reported that the vitamin-C was capable of ameliorating monosodium glutamate induced oxidative stress on renal function.

Conclusion

Lastly, we may conclude that the dimethoate, a commonly used organophosphate pesticide in the agricultural field that the produced hazardous alteration *i.e.* increased ACP, ALP, GOT, GPT and decreased protein levels in renal tissue. These alterations were more prominent in the later part of the experiment after dimethoate exposure as compared to control in different duration *i.e.* 30 and 60 days. All these changes may be induced by dimethoate directly or indirectly to tissues depending on dose and duration. But, these changes may be ameliorated by vitamin-C in *Clarias batrachus* overcome the stress of dimethoate and produced beneficial effects.

Fig.1 Showing the normal renal cell of control kidney *Clarias batrachus* (H&E X 400)

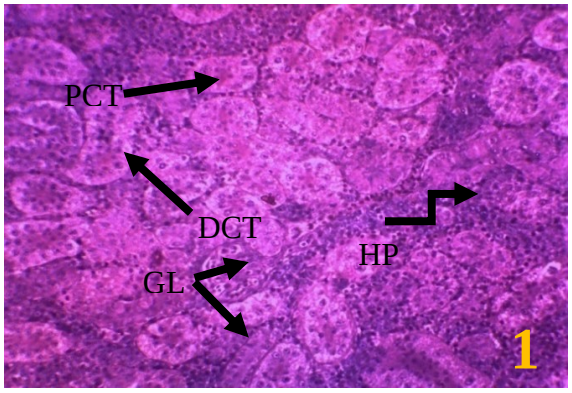
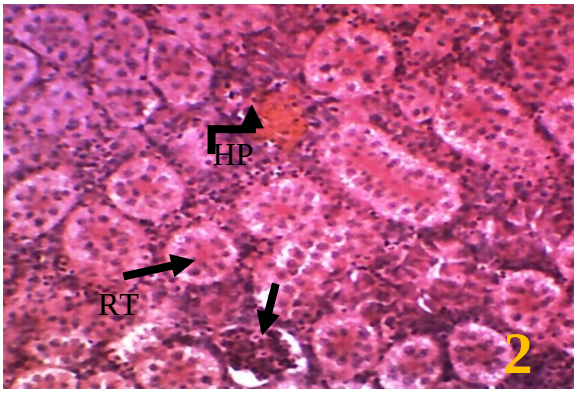
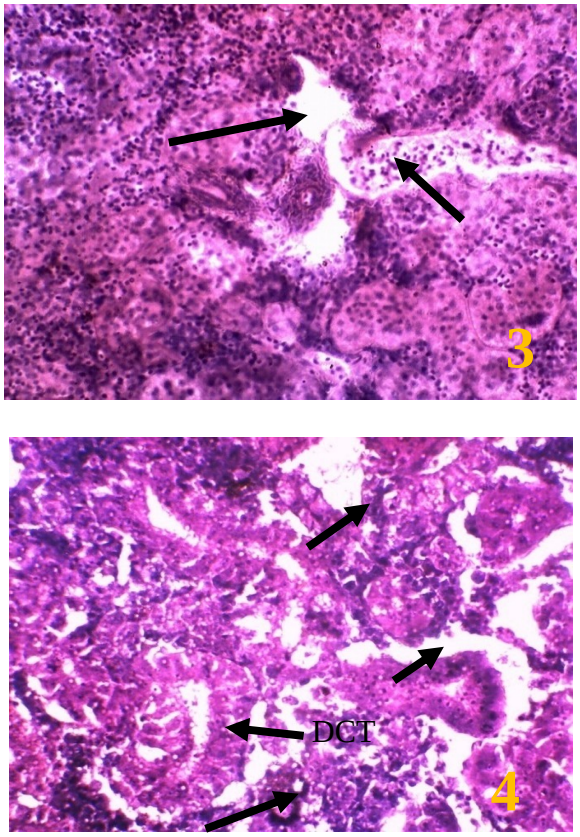


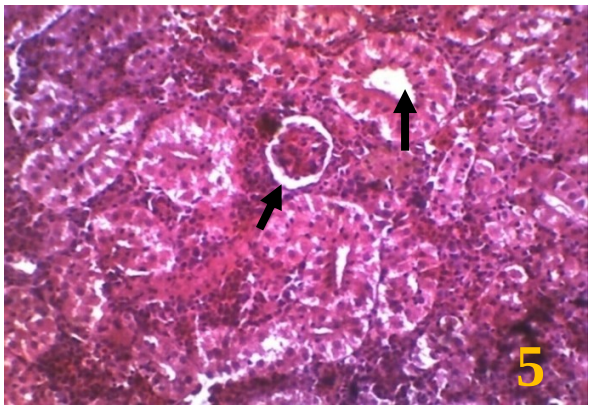
Fig.2 Section of kidney of fish, *Clarias batrachus* exposed to vitamin-C (50mg/l) for 30 and 60 days showing normal renal cells (R), glomerules and PCT, DCT tubules (H&E X 400).

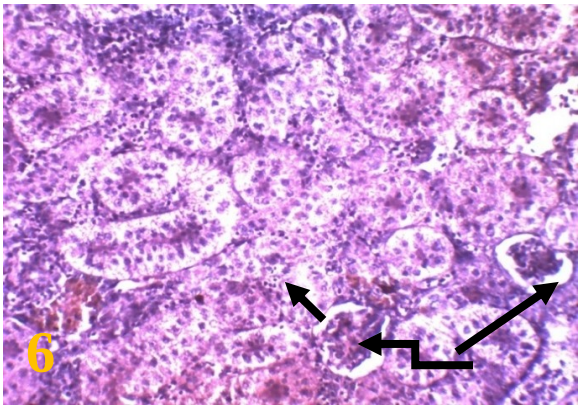


Figs.3&4 Section of kidney of fish, *Clarias batrachus* exposed to dimethoate (45µg/l) for 30 and 60 days showing infiltrated, the glomeruli were shrunken and atrophied with degeneration abnormal configurations with necrotic cells(NC), some cells appeared with damaged proximal and distal tubules(PCT, DCT) and marked atrophied glomerular cells (H&E X 400)

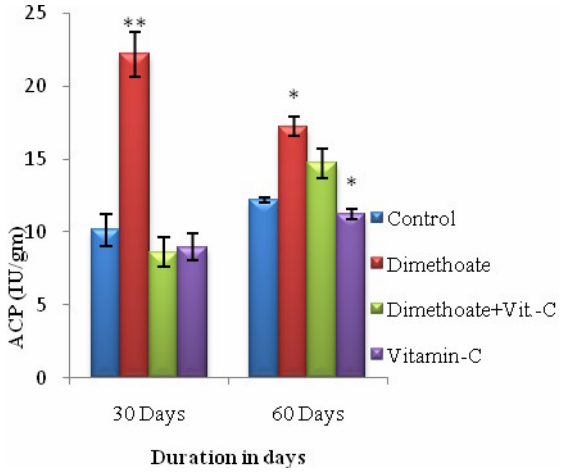


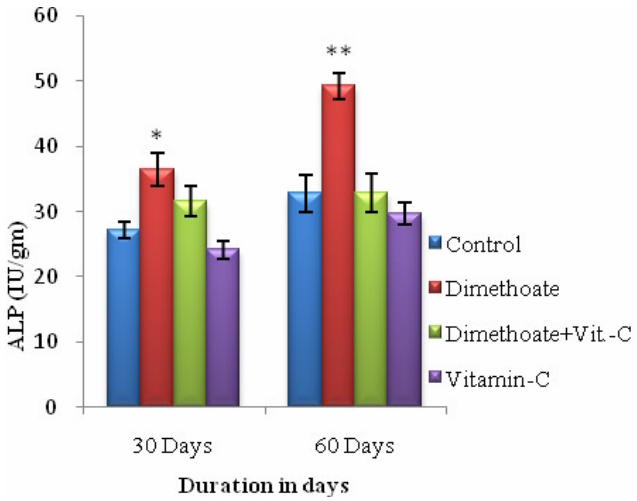
Figs. 5 & 6 Showing the recoveries renal cell of Kidney of *Clarias batrachus* exposed to Dimethoate + vitamin-C (45µg/l+50mg/l) for 30 and 60 days treated (H&E X 400)



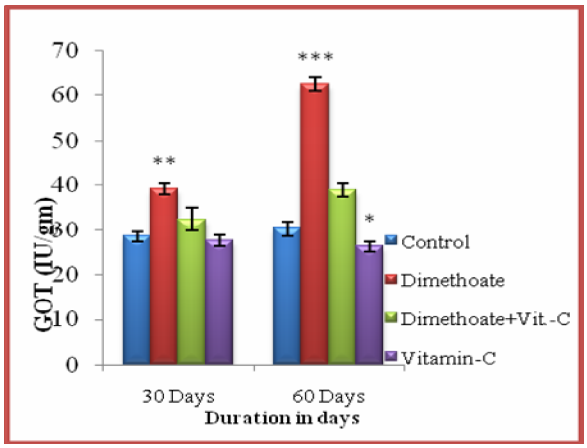


Figs.7 & 8 Acid phosphatase (ACP) and Alkaline phosphatase (ALP) activities (IU/gm) after different intervals i. e. 30 and 60 days treatment of dimethoate, dimethoate + vitamin-C supplemented, vitamin-C alone and control kidney of fresh water fish *Clarias batrachus*





Figs.9 & 10 Renal Glutamate Oxaloacetate Transaminase (GOT) and Glutamate Pyrophosphate Transaminase (GPT) activities (IU/gm) after different intervals i. e. 30 and 60 days treatment of dimethoate, dimethoate + vitamin-C supplemented, vitamin-C alone and control kidney of fresh water fish *Clarias batrachus*



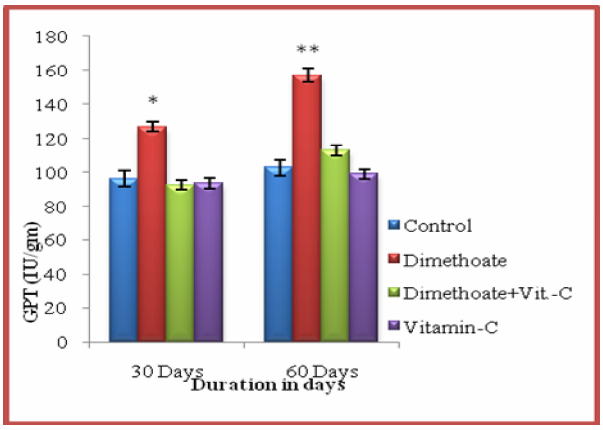
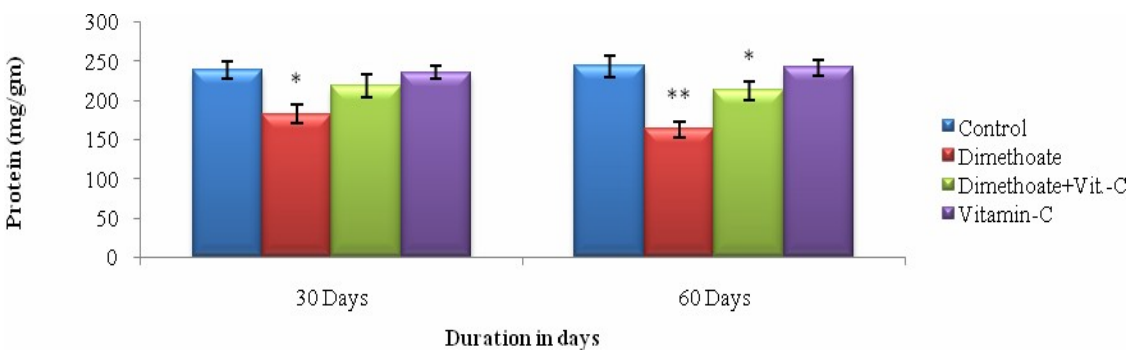


Fig.11 Protein estimation (mg/gm) after different intervals i. e. 30 and 60 days treatment of dimethoate and dimethoate + vitamin-C supplemented, vitamin-C alone and control kidney of fresh water fish *Clarias batrachus*



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