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NAPHTHALENE INDUCED ENZYMATIC ALTERATIONS IN THE LIVER OF CLIMBING PERCH, *ANABAS TESTUDINEUS*

Susri Nayak, Dipti Raut and Lipika Patnaik*

Environmental Science Laboratory, Department of Zoology, Ravenshaw University, Cuttack, Odisha- 753 003 India
**E-mail: lipika_pat@yahoo.co.uk*

Abstract: Polycyclic Aromatic Hydocarbon pollution has increased considerably in aquatic resources throughout the world and it is a growing global concern.The prolonged use of Naphthalene in many applications makes its way into the aquatic bodies, affecting the aquatic life. The climbing perch *Anabas testudineus* were exposed to different concentrations of naphthalene and the alterations in liver enzymes were assessed. The median lethal concentration of naphthalene to the fish *Anabas* for 72 hours was found to be 5.4mgl^{-1}.The activities of Aspartate Transaminase (AST), Alanine Transaminase (ALT), Acid Phosphatase (ACP), Alkaline Phosphatase (ALP) and Adenosine Triphosphatases (ATPases) were evaluated in the liver of both the control and experimental fishes. A major decrease was observed in all the enzyme activities in the liver of experimental groups when compared with the control group. Decrease in enzyme activity may be due to oxidative stress caused by naphthalene. Inhibited enzyme activity may also be attributed to organ dysfunction caused by naphthalene. The inhibition of enzyme activity was noticed to be dose dependent. The results indicate that alterations in liver enzymes can be used as a sensitive and potential biomarker for monitoring the health status of aquatic ecosystem.

Key words: Naphthalene, *Anabas*, Liver, Enzymes, Biomarkers

INTRODUCTION

Polycyclic aromatic compounds (PAHs) are a widespread class of chemical pollutants and known to exert acute toxic effects. Sources of PAHs include both natural and anthropogenic. Forest fires and volcanoes produce PAHs naturally whereas anthropogenic sources include incomplete burning of petroleum and its by-products, tobacco, automobile emissions, industrial and domestic wastewater, production of coke, carbon black, and coal tar (Kafilzadeh, 2015). Thus, PAHs are commonly detected in air, soil, and water. Therefore, PAHs are considered ubiquitous in the environment (Baklanov *et al.*, 2007; Latimer *et al.*, 2003). It has been proved that PAHs can cause carcinogenic and mutagenic effects and are potent immune-suppressants. Effects have been documented on immune system development, humoralimmunity and on host resistance (Armstrong *et al.*, 2004; CCME, 2010). PAHs enter the aquatic environment basically through oil spillage which greatly affects the aquatic biota. PAHs accumulate rapidly in aquatic animals, reaching greater concentrations than in the surrounding environment, which affects normal vital functions (Nagabhushanam *et al.*, 1991). PAHs affect the general functioning of the cells by damaging their membrane structure and the enzymes associated with it (Rengarajan *et al.*, 2015). PAHs of lower molecular weight usually hoard in greater concentrations in aquatic animals (Santos *et al.*, 2011). PAHs were ranked the ninth most threatening compounds to human health (Hossain *et al.*, 2010). Naphthalene ($C_{10}H_8$) is formed from two benzene rings fused together and is one of the intensively studied PAHs due to its high toxicity, low molecular weight and long persistence in water (Santos *et al.*, 2011). Naphthalene and metabolic by-products enter food chain through the systemic absorption leading to health issues. Naphthalene is found in mothballs and cleaning solutions. It is introduced in the environment mainly as a result of discharge from coal-tar production, distillation processes, spillage

of petroleum and by-products spillage. Naphthalene toxicity can cause skin irritation, headache, nausea, haemolysis, cataract, liver and kidney damage.

Fishes are the most exposed group of aquatic organisms to different types of toxicant due to deliberate addition of toxicants in the water bodies. Fish are relatively sensitive to any small change in the surrounding environment including the increase in pollution (Mary *et al.*, 2014).Fishes are at higher levels of food chain and accumulation of contaminants in fish biomagnifies the toxicants from water and hence widely used to evaluate the health condition of the aquatic ecosystem. *Anabas testudineus* is consumed as food largely by people in Odisha and is also recognized as good biological model due to its easy handling, maintenance in laboratory and for studying the possible changes due to toxic agents in toxicological assays. The toxic effects of PAHs are exerted on many organs; however liver is the target organ as biotransformation, detoxification and excretion of the toxic elements into bile is done in liver. Enzymes like AST, ALT, ALP and ACP are predominantly found in liver and serve as good indicators of stress condition in biological system as they play an important role in metabolism, detoxification and transamination reactions. Alkaline phosphatase is a hydrolase enzyme which removes various phosphate esters at an alkaline pH and mediates membrane transport whereas acid phosphatase is a hydrolytic enzyme which frees attached phosphate groups from other molecules at an acidic pH. Aspartate aminotransferase is responsible for transferring amino group form aspartate to 2- βglutamic acid forming glutamate and oxalo-acetate. Similarly, Alanine aminotransferase is responsible for transferring amino group from alanine to 2- ketoglutaric acid forming glutamate and pyruvate (Aruljothi, 2013). ATPases are membrane bound enzymes and exist in all cell membranes. They function as major carriers of chemical energy in all physiological activities and regulate ionic concentration of the cells. They are sensitive indicators of toxicity. Ca^{2+}-ATPase is significantly located in sarcoplasmic reticulum tubules and regulates the movement of Ca^{2+} ions from the cytosol to lumen of the sarcoplasmic reticulum and vice-versa to maintain low Ca^{2+} levels in the cell. Mg^{+}-ATPase enzymes are present in the mitochondrial membrane and play an important role in energy synthesis (Balaji et al., 2015). Na^{+} /K^{+} – ATPase is present in the plasma membrane of all animal cells and helps in maintaining resting potential, regulate cellular volume and in transport. The changes in the enzymatic system may alter the metabolic processes. The present study is carried out to study the lethal effects of Naphthalene onCa^{2+}-ATPase, Mg^{2+}-ATPase, Na^{+} /K^{+} – ATPase, ALT, AST, ALP and ACP in the liver tissues of *Anabas testudineus* and to evaluate whether the enzymes could be used as sensitive biological biomarkers in water polluted with PAHs.

MATERIALS AND METHODS

Fish specimen and maintenance

Fishes with the weight of 10±1 grams and length 10±0.5 cm were purchased from CIFA (Central Institute of Freshwater Aquaculture, Bhubaneswar). Fishes were stocked in glass aquariums and acclimatized in laboratory conditions for two weeks. The fishes were given dry commercial feed having 45% protein. Water was cleaned every day to remove excess feed and feacal matter.Water temperature, pH, dissolved oxygen, total hardness and alkalinity were regularly monitoredusing standard methods (APHA 1985).

Determination of LC_{50}

Initially toxicity tests were carried out to determine the median lethal tolerance limit of fish *Anabas testudineus* to naphthalene for 72 h. Separate glass aquariums having 50 L capacity were taken and different concentrations of naphthalene ranging from 0.1mgl^{-1} to 5.4 mgl^{-1} were introduced into the experimental tank.This was followed by release of 10 number of healthy fishes from the stock into each aquarium. They were starved for a period of 24 hours prior to the experiment. Duplicates were maintained for each concentration. At the same time, a control group (toxicant free) was also maintained in two different aquariums under identical conditions. The mortality of fish in control and experimental aquariums were recorded after a period of 72 hours. The median lethal concentration for 72 hours was found to be 5.4 mgl^{-1}. The number of dead fishes in the tank were removed and recorded immediately.

Short term toxicity studies

Ten aquariums with 50 L of capacity were taken and divided into five sets. Five sets were labeled as S-1, S-2, S-3, S-4 and S-5 and their duplicates were simultaneously maintained. S-1 served as the control (no dose) while S-2, S-3, S-4, S-5 were given dose concentrations of 4.4 $mgl^{-1,}$ 4.6 mgl^{-1}, 4.8 mgl^{-1} and 5 mgl^{-1} respectively. Six fishes were added to each aquarium and after 72 hours all the fishes were harvested and the livers were removed for the estimation of AST, ALT, ACP and ACP enzymes.

Assessment of ATPase activity

Liver tissues were washed in ice-cold normal saline (0.65%, W/V), blotted and stored at 20°C. 10% (w/v), homogenate of liver tissues was prepared in ice cold homogenizing 50 mM Tris–HCl buffer (pH 7.5) in a precooled porcelain mortar and pestle. The homogenate was centrifuged three times at 1000g for 10 mins, 10000 g for 20 mins and 10000 g for 5 mins. Then the final obtained supernatant was used for the estimation of Ca2+-ATPase, Mg2+-ATPase and Na+ /K+ – ATPase activities following the procedure of Takeo and Sakanashi (1985).

Sample preparation

The liver tissue was removed, cleaned and homogenized in chilled 0.9% ice cold saline solution. The homogenate was further centrifuged at 3000 rpm for 10 minutes and the supernatant was used for various enzyme assays like AST, ALT, ACP and ALP.

Estimation of acid and alkaline phosphatase

Acid Phosphatase and Alkaline Phosphatase were assayed following the methods of Andersh and Szaypinski (1974) and Bessey et al. (1946) using p-nitrophenyl phosphate as the substrate Phenol was used for plotting the standard graph. The values have been expressed in ì moles of phenol liberated / min / 100 mg of protein.

Estimation of Aspartate Amino Transaminase (AST) and Alanine Amino Transaminase (ALT)

Aspartate Amino Transaminase (AST) and Alanine Amino Transaminase (ALT) were spectrophotometrically assayed using SUN PHARMA kit. The colour developed was read at 520 nm against a reagent blank in Spectrophotometer.

Statistical Analysis

Data was analyzed using Student's t- test and expressed as mean± S.E. The p value of <0.05 was considered as significant against control. Statistically significant differences are indicated as *p <0.05.

RESULTS

Mortality of the fish was not noticed during acclimatization period. Similarly, no diseased condition was observed during both acclimatization period and toxicity test periods.The average mean values of water quality during experiment was pH 7.56 ± 0.10, temperature 22.60 ± 0.39 °C, dissolved oxygen 5.12 ± 0.57 mgL^{-1}, total hardness 161.2 ± 0.87 mgL^{-1} and alkalinity 146.4 ± 0.76 mgL^{-1}. In the present study the 72 hour LC_{50} was found to be 5.4 mg/l. For acute toxicity tests naphthalene concentrations of 4.4 mgl^{-1}, 4.6 mgl^{-1}, 4.8 mgl^{-1} and 5 mgl^{-1} were taken and no mortality was recorded during the test period.

Fig. 1 shows the Na^+/K^+-ATPase activity of liver tissue exposed to different concentrations of naphthalene for 72 hours. Results show significant decrease in Na^+/K^+-ATPase activity in all the experimental set-ups as compared to control.Inhibition of Na^+/K^+-ATPase activity in liver tissues ranged from 13.64% - 57.37%. Fig. 2 provides the Mg^{2+}-ATPase activity liver tissue of *A. testudineus* exposed to different concentrations of naphthalene for 72 hours. There was a significant decrease in Mg^{2+}-ATPase activity in all the experimental fishes as compared to control fishes. A decrease of 13.53% - 71.92% was noted in the liver tissues exposed to naphthalene. Fig. 3 represents the Ca^{2+}-ATPase activity in the liver tissues of *Anabas testudineus* exposed to different concentrations of naphthalene. A decrease of maximum 65.67% was seen in liver tissues of experimental fishes.

Fig. 4 and Fig. 5 shows the ALT and AST activity of liver tissue exposed to different concentrations of naphthalene for 72 hours.A significant ($p < 0.05$) inhibition of enzyme activity was noticed when compared to the control.Results indicated an inhibition of 16.12% - 38.70% as compared to the ALT activity of control fishes. Similar results were also seen in aspartate aminotransferase (AST) activity when the enzyme activity of experimental fishes was compared with control fishes. An inhibition of 9.56% - 37.56% of enzyme activity was noticed.

Fig. 6 and Fig. 7 presents the data on changes in the

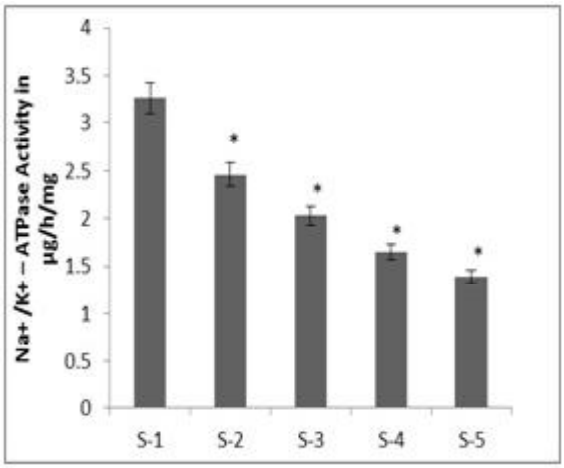
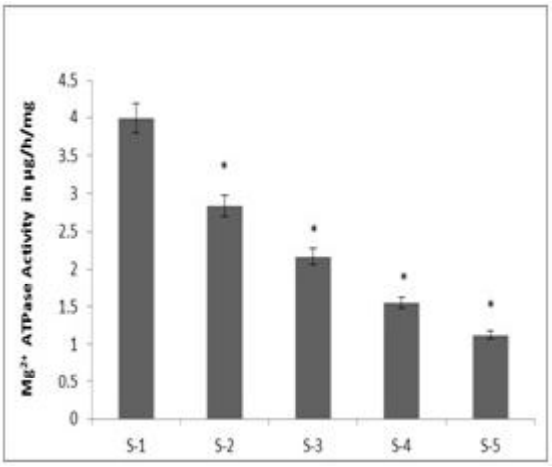


Fig. 1.



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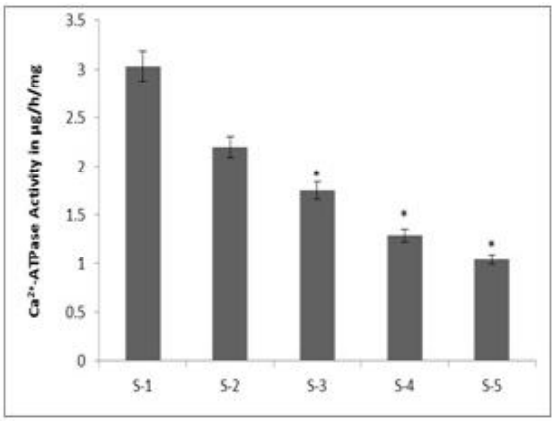

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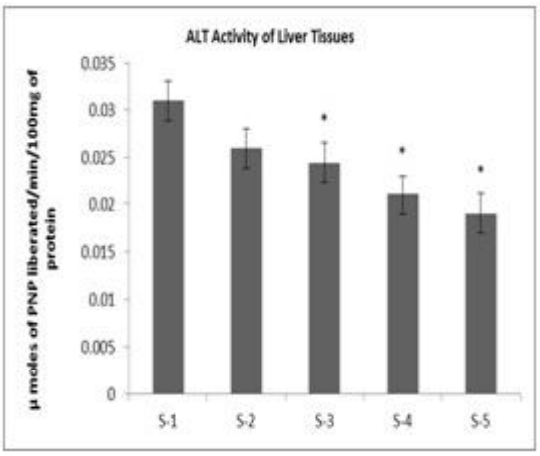

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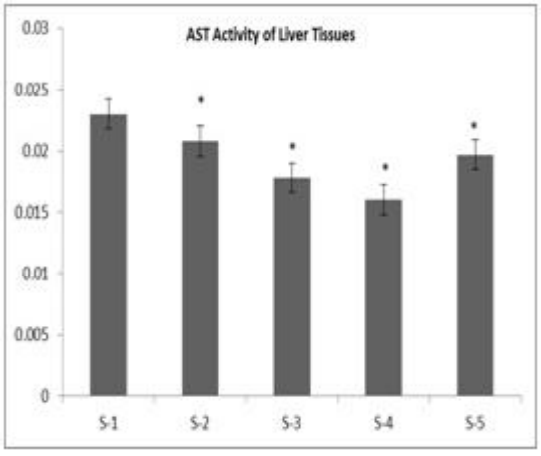

Fig. 5.

Figs. 1-3. Inhibition of ATPase activity in *Anabas testudineus* exposed to different concentration of naphthalene for 72 h, S-1 (Control) S-2 (4.4 mgl^{-1}), S-3 (4.6mgl^{-1}), S-4 (4.8 mgl^{-1}) and S-5 (5 mgl^{-1}). (1) Liver Na^+/ K^+-ATPase level (µg/h/mg), (2) LiverMg^{2+}-ATPase level (µg/h/mg), (3) Liver Ca^{2+}-ATPase level (µg/h/mg)Values are mean±S.E. of six individual observations. *Significant at $p < 0.05$ (based on t-test).

Fig. 4-5. Inhibition of ALT and AST in *Anabas testudineus* exposed to different concentrations of naphthalene for 72 h, S-1(Control) S-2 (4.4 mgl^{-1}), S-3 (4.6 mgl^{-1}), S-4 (4.8 mgl^{-1}) and S-5 (5 mgl^{-1}). (4) Liver ALT level (µ moles of PNP liberated/min/100 mg of protein), (5) Liver AST level (µ moles of PNP liberated/min/100 mg of protein). Values are mean±S.E. of six individual observations. *Significant at $p < 0.05$ (based on t-test).

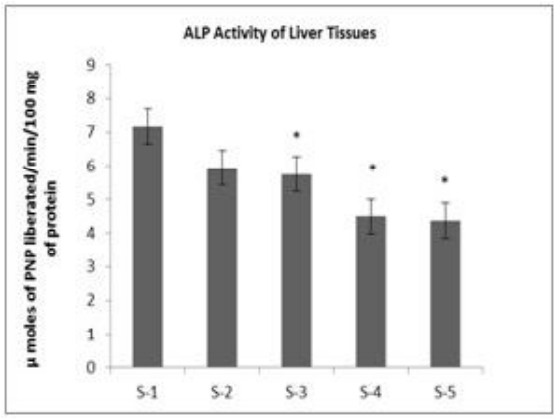

Fig. 6.

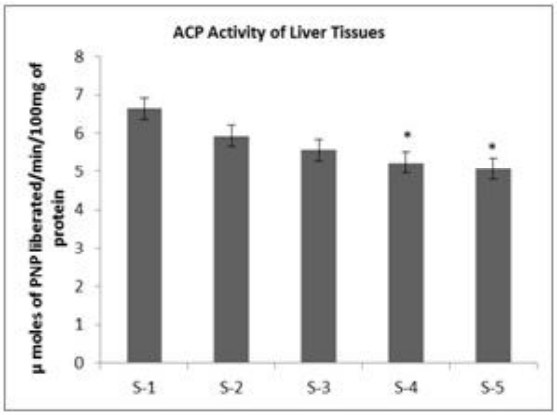

Fig. 7.

Fig. 6-7. Inhibition of ALP and ACP in *Anabas testudineus* exposed to different concentrations of naphthalene for 72 h, S-1 (Control) S-2 (4.4 mgl^{-1}), S-3 (4.6mgl^{-1}), S-4(4.8 mgl^{-1}) and S-5(5 mgl^{-1}). (6) Liver ALP level (µ moles of PNP liberated/min/100 mg of protein), (7) Liver ACP level (µ moles of PNP liberated/min/100 mg of protein). Values are mean ± S.E. of six individual observations. *Significant at $p < 0.05$ (based on t-test).

ALP and ACP activity of liver tissues exposed to different concentrations of naphthalene for 72 hours. Liver ALP and ACP activity inhibited significantly ($p < 0.05$) compared to control values at the end of exposure.The inhibition of ALP enzyme activity in liver tissues of experimental *Anabas* varied from 17.15% to 39.05%. The acid phosphatase activity was found to be decreased significantly by 10.69% - 23.79% as compared to control.

DISCUSSION

Estimation of various enzymes like ATPase, ALP, ACP, AST, ALT in liver tissue of *Anabas testudineus* in response to Naphthalene showed decline in all the concentrations as compared to the control. Inhibition of enzyme activity increased with increase in dose concentration and it might be due to oxidative stress (Vijayavel *et al.*, 2006; Sogbanmu *et al.*, 2018). It also indicates serious pathological damage caused due to naphthalene exposure (Sunmonu *et al.*, 2009). Studies have shown that petroleum products and PAHs are responsible for decrease in enzyme activities of aquatic animals (Shirmohammadi *et al*., 2017; Sunmonu *et al.*, 2015; Vijayavel *et al.*, 2006; Elumalai and Balasubramanian, 1998). PAHs also affect the health of human beings as fishes form the basic form of our protein diet.

Enzymes have been widely used as indicators of pollution in the environment. ATPases are found in all cell membranes and regulate ionic concentration in the cells (Balaji *et al.*, 2015). In our present investigation it was noticed that the Na^+/K^+-ATPase activity of liver exposed to different concentrations of naphthalene decreased significantly. Results also indicated that inhibition % increased with increase in dose concentration. Na^+/K^+-ATPase is a membrane bound enzyme involved in active transport of Na^+ and K^+ across cell membranes and thus helps in maintaining the membrane potential and osmotic equilibrium of cell. Toxicants can disrupt the the Na^+/K^+-ATPase activity by interfering with their metabolic pathways or by direct interfering with the the enzyme complex, inducing allosteric change resulting in inhibition of ATPase activity. Similar inhibitory results were also seen in the liver, gills and muscles of *Clarias gariepinus* exposed to potassium cyanide (Oseni, 2015). Decrease in Na^+/K^+-ATPase activity of gills was also noticed in *Cirrhinus mrigala* when exposed to toxicant silver nitrate (Sathya *et al.*, 2012). Any change in the ion concentration due to decreased Na^+/K^+-ATPase activity may cause the cell to rupture or shrink ultimately harming the organism.

Mg^{2+}-ATPase enzyme is associated with mitochondria and plays an important role in energy synthesis of ATP by coupling ADP and Pi. Decrease

in Mg^{2+}-ATPase enzyme in the liver tissues is indicative of disturbance in energy yielding process by disrupting oxidative phosphorylation. Ca^{2+}-ATPase is required for the maintenance of calcium ions in the cells. Decrease in Ca^{2+}-ATPase will increase the number of calcium ions in the cell thus imbalancing the physiological state of the cell. Decrease in Mg^{2+}-ATPase activity was also recorded when *Anabas testudineus* was exposed to lead (Afsar *et al.*, 2012). Literature also reveals that aquatic toxicants like lead inhibits Mg^{2+}-ATPase and Ca^{2+}-ATPase activity in muscles and gills of *Oreochromis niloticus* (Dogan *et al.*, 2015). Decrease in Ca^{2+}, Mg^{2+} and Na^{+}/K^{+} - ATPase activity was also noticed in *Cyprinus carpio* exposed to Cypermethrin. Phenolic compounds like phenol and m-cresol also inhibited in Ca^{2+}, Mg^{2+} and Na^{+}/K^{+} - ATPase activity in liver and of *O. mossambicus* (Varadarajan *et al.*, 2014). Any change in ATPase enzymes activity from the normal range wills imbalance the ionic concentration of the cells and affect the normal functioning of the animal body.

Decrease in ALP, ACP, AST and ALT activities in the liver of *Anabas testudineus* exposed to naphthalene could be either due to their possible leakage from the cytosol into general blood circulation through damaged plasma membrane or decrease in their synthesis as a result of organ dysfunction. The decrease was concentration dependent and could be the manifestation of oxidative stress caused by naphthalene (Sunmonu *et al.*, 2015). Necrosis of liver tissue and uncoupling of oxidative phosphorylation might be responsible for the decrease and inhibition of acid phosphatase (Magar and Shaikh 2013). Investigatory studies on ACP and ALP have been carried out by exposing female fiddler crabs to varying concentrations of cadmium and similar results have been observed. Decrease in ALP enzyme activities probably would facilitate the increased activity of phosphorylation enzyme in the tissue of fish and cause subsequent break down of glycogen for energy release during toxic stress(Suresh *et al.*, 2016). Similar reports in *Clarias gariepinus* indicates fall in the rate of synthesis of glycogen resulting from low metabolism demand and decrease in metabolic transport due to decreases in ALP (Evelyn *et al.*, 2013). Our results are in complete agreement with studies made on changes in ACP and ALP in the liver and kidney of *Labeo rohita* exposed to high concentrations of heavy metal (Mir *et al.*, 2016). The result showed decreased enzyme activity due to the direct binding of the metal with enzyme protein or the toxic effects produced by them on tissues leading to decreased synthesis of enzymes. A reduction of 20% and 61% in liver ACP and ALP was also noticed when *Oreochromis mossambicus* was exposed to organophosphorus insecticide (RPR-V) (Venkateswara, 2006). However our results are not harmonious with the observation of where increased activity of ALP enzyme was recorded in *Tilapia* exposed to butylbenzylphthalate (Sepperumal and Saminathan, 2014). Decrease in ALP enzyme activity may result in altered transport, inhibitory effect on the cell growth and proliferation whereas decrease in ACP may be due to increased glycogenolysis or changes in the mitochondrial membrane function (Sreekala and Zutshi, 2010). Decrease in ALP, ACP, AST and ALT enzyme activities have also been noticed in *Channa punctatus* when exposed to chromium trioxide for different time periods (Pal and Trivedi, 2016). Decreased level of ALT and AST were also noticed when muscles of *Oreochromis niloticus* were exposed to heavy metals. The decreased activities of ALT and AST indicate disruption in the structure and integrity ofcell organelles, like endoplasmic reticulumand membrane transport system (Yacoub and Gad, 2012). Decrease of AST enzyme activity in muscles was also recorded when *Channa punctatus* was exposed to different concentrations of copper sulphate. Decrease in enzyme activity may be the result of toxicants accumulating in the liver in increasing concentrations (Nathiya *et al.*, 2017). Decrease in ALT and ALP enzyme activity was also noticed in *Heterobranchus bidorsalis* exposed to another PAH anthracene (Sunmonu et al., 2009). ALT and AST are highly concentrated in liver and act as markers of healthy liver functioning. An change in the enzyme activity clearly states liver dysfunction due to the stress caused by Naphthalene. Decreased synthesis of enzymes in our study may be due to hepatic damage and necrosis of hepatocytes by naphthalene (Bakde and Poddar, 2011). Damage to the hepatocytes may also lead to the leakage of the enzymes into the general blood

circulation. Hence decreased activity of all liver enzymes is a sensitive biomarker of naphthalene toxicity. Decreased enzyme activities in the present study may be due to disruption of normal functioning of liver which may lead to the maiming of the detoxification processes due to naphthalene. However, the degree of inhibition of different enzyme activity may vary from species to species indicating that the exposure duration, dose, ecological conditions and the toxicant play an important role in the inhibition of various enzymes.

CONCLUSION

The present study clearly indicated that naphthalene inhibited enzyme activity in liver tissues of *Anabas testudineus*. It can be concluded that naphthalene is a toxic agent which interferes with various biochemical activities thus imbalancing the physiological status of the body. Thus, these enzymatic parameters can be used as environmental biomarkers for monitoring naphthalene toxicity/ pollution in aquatic ecosystem. As fish forms a major part of protein diet in human population, the discharge of naphthalene into aquatic bodies can be guarded and remediation techniques can be developed to avoid naphthalene pollution.

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