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UREA ENDUCED BLOOD METABOLIC & HISTOPATHOLOGY CHANGES IN VITAL ORGANS OF *Anabas testudineus* (Bloch.)

Deep Gupta (Research scholar),

Dr. Arti Kumari,

Department of Zoology, C.M.Sc. College, L.N.M.U., Darbhanga
Email: deepgupta1196@gmail.com

ABSTRACT

The current study includes the blood metabolite and histopathological alterations induced by chronic (20 days) exposure of the fish Anabas testudineus to a sublethal concentrations (27.5 mg/L) of inorganic fertilizer, Urea [$CO(NH_2)_2$]. Significant changes were observed in the blood biochemical properties in the form of hyperglycemia, hyperproteinemia and hyperchlosterolemia. Some vital organs were taken for the histopathological study that is kidney, liver, intestine, testis and ovary. The present study showed major histopathological alteration like vacuolar degenerative changes, necrosis etc. found in liver, intestine, testis, ovary and kidney organs dysfunction in response to urea toxicity effect in the fish A. testudineus. So, it is suggested that more suitable to culture at water fertilizer, Urea concentration of < 27.5 mg/l for optimum growth performance. The information will be major role on different levels of responses of organisms with respect to pollutant stress is a necessary pre-requisite for the proper management of fertilizer application in agriculture and aquaculture.

Key words: *Anabas testudineus*, blood metabolite, fertilizer, histopathology, pollutant, Urea.

INTRODUCTION:

Over the years, different types of chemical fertilizers are used to increase crop production in intensive farming. However, these fertilizers have caused both lethal and sub-lethal side effects to aquatic life when they dissolved and washed into fresh water ecosystems through rains (Omoregie *et al.,* 2009; Ajima *et al.,* 2015). For nitrogen

source, Urea [$CO(NH_2)_2$] is one of the most widely used fertilizer in India. Urea when mixed with water body, it was found to diminish fish production and also cause mortality (Jhingran, 1982).

Histopathological studies on aquatic fauna are a noteworthy and promising field to understand the structural organization that occurs in the organs due to presence of pollutants in the aquatic bodies. These histological structure changes vary with the body parts-organs, nature of the pollutant, medium and duration of exposure. Water physio-chemical characteristics also influence histopathological manifestations of toxic effects (Galat *et al.*, 1985).

Anabas testudineus (Bloch.), locally known as "kawai", which is an integral part of paddy field culture on this subcontinent, is also subjected to severe ammonia toxicity from ammonium fertilizers during the intensive fertilization of the crop fields.

Hence, in this paper efforts have been made to illustrate the blood metabolites and histopathological alterations induced by this inorganic fertilizer, urea on the liver, kidney, intestine and gonads toxicity impact on air breathing teleost, *Anabas testudineus.*

MATERIALS & METHODS :

The air-breathing teleost *Anabas testudineus* (Bloch.) procured and brought in container live from the local fish market, Darbhanga were washed with 0.1% $KMnO_4$ solution to remove dermal infection if any. Healthy fish of average length (10–12 cm) and weight (30–34 g) were acclimated for 15 days to laboratory conditions. Commercial diet containing 28.58% crude protein was used through the experiment period with daily ration rate 3% of fish weight in the in morning (10.00 AM). Running tap water was used in all the experiments and the fish were adjusted to natural photoperiod and ambient temperature. No aeration was done and follows the methods of APHA (1985).

Static acute bioassays were performed to determine LC_{50} values of urea, the mortality was recorded after 24, 48, 72 and 96 hr, and were calculated by the Finney method (1978). The LC_{50} values for these periods were 209 mg, 221 mg, 240 mg and 275 mg respectively. 1/10th value of the LC_{50} value for 96 hr was taken as the sublethal concentration (Sprague, 1971). Twenty acclimated fish were exposed to a sub-lethal concentration (27.5 mg) of urea for 20 days. Side by side same number of fish as that of experimental one was maintained as the control groups. At the end of exposure period the fish were anaesthetized with 1:4000 MS 222 (tricane, methane, sulfonate, sandoz) for two minutes. On 20^{th} day blood samples was extracted from the caudal dorsal of the test fish and were then processed for quantitative estimation of blood glucose (Sinha, 1990), serum protein ('Biuret method' of Varley *et. al.,*1980) and serum cholesterol (Kabara's method,1966) and same day fish were taken out, sacrificed and the liver, intestine, kidney, testis and ovary were excised out and fixed in 10% Neutral Buffered Formalin for 18-24 hours fixed tissue samples were then processed and paraffin embedded tissue

blocks were cut into serial sections (5-7 µ thick) by a rotary microtome and all the tissues was prepared using the standard histological methods (Luna, 1968), stained with Haematoxylin and Eosin and microphotographs were taken.

RESULTS:

BLOOD METABOLITE LEVELS: The variables monitored at blood metabolites levels are blood glucose, serum protein and serum cholesterol of experimental fish *A. testudineus.* On exposure to a sub-lethal concentration (27.5 mg/l) of urea the fish reveals following changes in the biochemical parameters.

BLOOD GLUCOSE: The blood glucose level in the control fish group is assessed to be 68.66 ± 0.33 mg/100ml of blood. The experimental fish become hyperglyceric as evident by a highly significant ($p < 0.001$) elevated level of glucose in the blood (88.82 ± 1.41 mg/100ml) which counts to an increase of 28.53% (Table-1) the present values of the present observations are expressed as meant S.E. of 5 fish in each group.

SERUM PROTEIN: The experimental fish group showed highly significant ($P< 0.001$) depletion in the level of serum protein in control group estimated to be 6.01 ± 0.37 g/100 ml as against 3.73 ± 0.15 g/100 ml in the treated group (Table-1).

SERUM CHOLESTROL: The serum cholesterol level in the fish of control group has been analysed 202±2.08 mg/100 ml. The treated fish show hypercholestrolemic response as evident by significant ($p< 0.05$) increase (228.8± 1.96 mg/100 ml) in its level (Table-1) the serum cholesterol has been found to increase by 13.03% in the present case.

Tables:-1

Changes in the blood / serum metabolite levels in *Anabas testudineus* exposed to urea (27.5 mg/l) for 20 days. Values are mean ± SE of 5 observations.

Parameters	**Control**	**urea exposed**
Blood glucose (mg/100 ml)	68.66±0.83	88.82± 1.41(+28.53)
Serum protein (g/100ml)	6.01 ± 0.37	3.73 ± 0.15 (- 37.90)
Serum cholesterol (mg/100 ml)	202±2.08	228.8±1.96 (+13.07)

Values indicate percent increase (+)

Or decrease (-) over control values significant at

* *P<0.05,* *** *p<0.001*

HISTOPATHOLOGY: Tissue samples liver, kidney, intestine, testes and ovary of *A. testudineus* were treated with sublethal urea concentration 27.5 mg/l at 20 day showed following major significant results:-

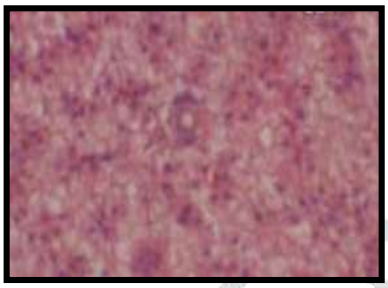

Figure- 1.A.– Photomicrograph of the normal liver of control fish, *Anabas testudineus*. H. & E., 100X.

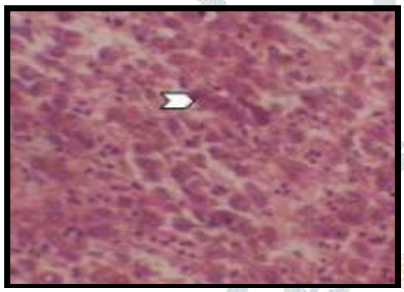

Figure-1.B. – Photomicrograph of the liver of *Anabas testudineus* treated with urea- 27.5 mg/L for 20 days showing hemorrhagic liver tissue (⇨), blood congestion and necrotic cells. H. & E., 100X.

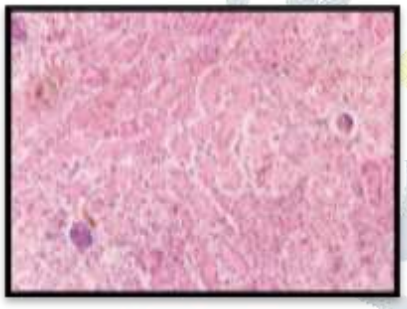

Figure:-2.A.-Photomicrograph of kidney of *Anabas testudineus* from control group showing normal. H.&E., 200X.

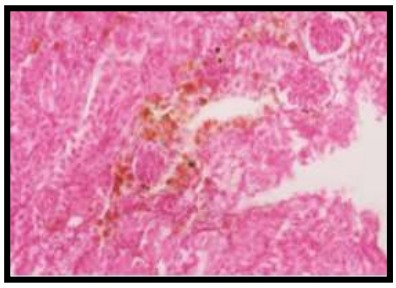

Figure:- 2.B.- Photomicrograph of kidney of *A. testudineus* treated with urea 27.5 mg/l for 20 days showing degeneration of renal tubular epithelium, vacuolation and necrosis of renal tubules along with infiltration and necrosis of melanomacrophage center (arrow). H.&E., 20X.

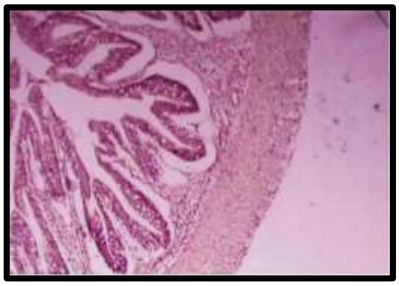

Figure:-3.A.-Photomicrograph of Intestine tissue of *A. testudineus* in control groupshowing normal appearance of circular muscles, longitudinal muscles, serosa and villi. H.&E., 120X.

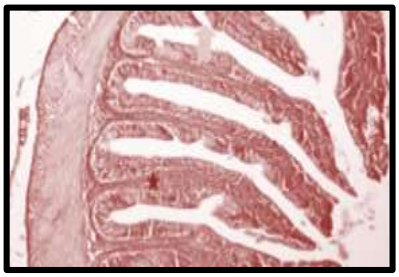

Figure:-3.B.- Photomicrograph of Intestine tissue of *A. testudineus* exposed to urea 27.5 mg/L for 20 days showing desquamation (orange arrow) and mononuclear cell infiltration (MHI) (arrow). H.&E. 120X.

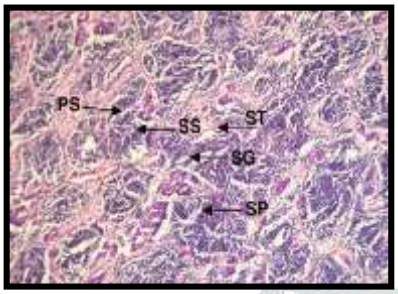

Figure- 4.A. – Photomicrograph of the testes of *Anabas testudineus* control fish showing sperm (SP), spermatogonia (SG), spermitide (ST), secondary spermatocyte (SS), primary spermocytes (PS). H.&E., 200X.

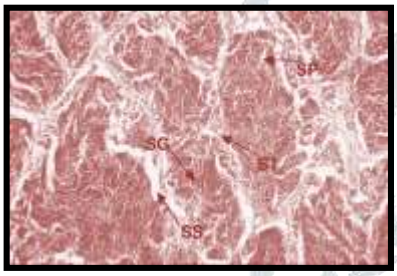

Figure- 4.B. – Photomicrograph of the testes of *Anabas testudineus* treated with urea 27.5 mg/L for 20 days showing sperm (SP), spermatogonia condensation (SG), spermitide (ST), secondary spermatocyte vacuolation (SS). H.&E., 200x.

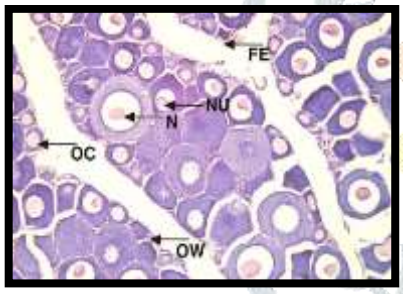

Figure- 5.A. – Photomicrograph of the ovary of *Anabas testudineus* control fish showing (OW) Ovarian wall, (FE) Follicular epithelium, (N) Nucleus, (NU) Nucleolus, (OC) Oocyte. H.&E., 200X.

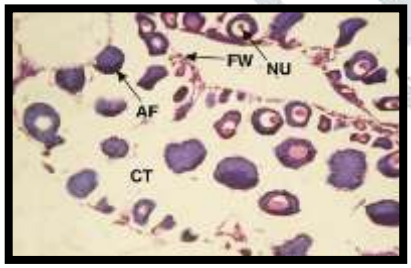

Figure 5.B. – Photomicrograph of the ovary of *Anabas testudineus* treated with urea 27.5 mg/L for 20 days showing (NU) Nucleolus condensed, (CT) Connective tissue degenerate (AF) Atretic follicle & (FW) Follicular wall disrupted. H.&E., 200X.

LIVER: Histology of control fish groups were in normal structure. The liver is composed of hepatic lobule in which the central vein obscure. The parenchyma of the hepatic lobule is formed from hepatocytes which are arranged around the blood sinusoid in cord-like structure known as hepatic cell cord. There are bile ductile in between the cord of hepatic cells which are directed toward the periphery of the lobule to open in the bile duct (Figure:-1.A.). *Anabas testudineus* exposed to sub-lethal concentrations of urea - 27.5 mg/L for 20 days showed varied degree of hepatic cirrhosis as evidenced by vacuolization, space formation and resulting haemorrhage, vacuolar degeneration, necrosis, hyperemia and mononuclear cells filtration in portal regions were observed (Figure:-1.B.).

KIDNEY: The fish kidney consists of head and body kidneys. The head kidney is the anterior portion of the kidney and consists of lymphoid tissue. The epithelium becomes lower and more cuboidal in the intermediate segment. The distal convoluted tubules have epithelium with lightly eosinophilia and have no brush border (Figure:-2.A.), and in urea exposed fish's kidneys displayed glomerulonephritis, vacuolar degenerative changes in the tubular epithelium and slight congestion (Figure:-2.B.).

INTESTINE: The intestinal wall of control fish, *A. testudineus* comprised of four distinct layers, viz. mucosa, submucosa, muscularis and serosa. The mucosal layer being thrown into finger like villi, which is made up of simple, long columnar cells and numerous goblet cells (mucous cells) with centrally placed nuclei. Sub-mucosa is thin and projected into mucosal folds constituting the lamina propria. This layer is composed of loose connective tissue with numerous collagen fibres and blood cells. Muscularis consists of inner, thick, circular, and outer, thin, longitudinal muscular layers. Serosa is formed of peritoneal layer and blood capillaries (Figure:-3.A.). In urea exposed, marked histopathological changes in the intestine of *A. testudineus* have been observed in intestinal tissue, hydropic degeneration, necrosis and desquamation in epithelium cells at the apex of the villi were determined and mononuclear cell infiltration in the lamina propria was slightly observed (Figure:-3.B.).

TESTES: Histology of normal testes shows the presence of healthy seminiferous tubules, which is internally lined by tubular epithelium which gives rise to spermatocytes. Testis of control fish were composed of lobules showing active spermat ogenesis. Sperm nests were found in majority of lobules (Figure:-4. In testes the seminiferous tubules are normally of varying shapes and sizes, each tubule has a definite thin fibrous wall which is not distinguished after spawning. It shows reduction in the number and condensation of spermatogonic cells as well as inflammation of cells, contraction and vacuolation of tubules (Figure:-4.B.).

OVARIES: Histology of control fish have thick ovarian wall with increased vascular supply and conspicuous blood capillaries. The connective tissue in the stromal was evident in good volume. The germ cells become associated with small epithelial cells more into cortex. The associated epithelial cells multiply and surround the germ cell which is now called oocyte developing into the stage I, stage II, stage III etc. and they will develop into the mature ovum which is nourished by the surrounding follicular cell (Figure:-5.A.). Follicular cells are disrupted. Nucleolus shows condensation of crescent shaped dark granules at one side. Degeneration of epithelial cells causes vacuolation, breakdown of germinal vesical, many disrupted oogonia are the changes caused due to the exposure of ovary of *A. testudineus* to sublethal dose of urea showing in (Figure:-5.B.).

DISCUSSION:

The results were observed shows similarity of earlier workers, here we discussed. The present investigation was undertaken to study the blood metabolite and histopathological changes occurring in the some vital organs of *Anabas testiduneus* after exposure to sublethal dose of urea- 27.5 mg/L. Urea an effect is known to increase the

levels of activating glycogenolysis and glyconeogenesis with a net result of increasing plasma glucose levels. These results confirm the corticosteroid response to high ammonia observed by Davis *et al.* (2003) reported that High NH_3-N concentration caused a significant increase in plasma glucose concentrations in both PC and isoeugenol treated catfish. Recently Singh and Chaudhary (2011), Koley and Kumar (2012) and Zhang *et al*., (2017) has observed similar result under the exposure of Nuvan, fenvalerate and Eklaux in various fresh water fishes.

The decrease in serum protein as observed during the present study suggests that the detoxification/ degradation of the toxicant either took place partially in the blood itself or involved the serum protein. This fall further suggests a progressive protein degradation or biochemical transformation of the protein Nitrogen into other nitrogenous products as suggested by Singh and Chaudhary (2011), Koley and Kumar (2012), Ahsan (2016) and recently Pathak, (2020) has observed similar result under the exposure of Nuvan, fenvalerate, Atrazine and Eklaux in various fresh water fishes.

The present investigation exhibited hypercholesterolemia (Table-1) under the toxic influence of urea and in agreement with the findings of Singh and Chaudhary (2011), Koley and Kumar (2012), Rani, *et al.*(2015), Dilip, and Vidya (2016) and Pathak, (2020) has observed similar result under the exposure of Nuvan, fenvalerate and Eklaux in various fresh water fishes.

HISTOPATHOLOGY:

The tissue samples liver, kidney, intestine, testes and ovary of air breathing fish *Anabas testudineus* were treated with sublethal urea concentration 27.5 mg/l at 20 day after sacrificed. The liver of *A. testudineus* in the present study showed group exposed to the urea showed hyperplastic hepatic and necrosis of hepatic cells. Similar observations were made in findings by Kalaiyarasi, (2017). Liver is the major metabolic center and any damage to this organ would subsequently do, so many physiological disturbances leading to subsequent mortality of fish (Mishra & Poddar (2016). The Necrosis in the liver could be due to the extra work load on hepatocyte during detoxification of the cypermethrin (Ullah, *et al*. 2015). Recently same effects observed in application of ammonium chloride on fish *Clarias batrachus* by Sangeeta, *et al.*, (2020). The above studies support the present experiment with urea.

In our study, kidney tissues displayed glomerulonephritis and hyperemia after being exposed to different concentrations of sublethal ammonium chloride concentrations where the kidney is a one of the major organs of the toxic effects. Thurston *et al.,* (1978) observed hydropic degeneration in the kidney of trout after exposure to 0.34 mg /1 NH_3–N. Intracellular vacuolation, necrosis and shrinkage of nuclei were also apparent in the present study in urea treated *Anabas testudineus*. Nayan (2012) reported that degeneration of renal tubule epithelia, hyaline droplet degeneration, eventually may induce renal failure. Tilak, *et al.,* (2001) also reported same in *Ctenopharyngodon idellus*. The above reporting is similar to the present observation.

Histological analysis of the digestive system is considered a good indicator of the nutritional status and toxicant ingestion of fish (Caballero *et al.*, 2003). The proliferation, necrosis of serora and mucosa and rupture of villi have been reported by Sastri and Gupta (1978) in *Channa punctatus*; Kumar and Pant (1984) in *Barbus conchonius*; against exposure to heptachlor, zinc and copper salt mercuric chloride, dimecron, aldicarb and furadan, respectively. The above reporting is similar to the present work.

Degeneration of epithelial cells causes vacuolation, breakdown of germinal vesical, many disrupted oogonia. Maximum damage is produced exposure of urea in the ovaries of *Anabas testudineus*. The histological abnormalities in ovaries may be caused by several factors viz. ionizing radiations, electric current, parasitic infections, xenobiotic toxicants and by a variety of effluents and aquatic pollutants (Shukla *et al*., 1984), heavy metal on *Cyprinus carpio* by Vinodhini *et al.* (2009); fertilizer, ammonium chloride on fish *Clarias batrachus* by Sangeeta, *et al.*, (2020). The above reporting is similar to the present findings.

Testicular inflammation was documented as one of the common responses in both aquatic and terrestrial animals exposed to environmental toxicants (Sokal *et al.*,1985), in term of vacuolization of tubular cells and distortion of seminiferous cells along with inflammatory lesions. The degenerative changes in seminiferous tubules, enlarged interstitium and hemorrhage in intertubular area in albino rats exposed to pesticides have been reported. Baronia and Sahai (1993). Zutshi (2005) observed the effect of fenthion on the testes of *Glassogobius giuris*. Lata, *et al.*, (2008) observed reduction in size with spermatids and sperms in degenerating condition. Shyni & Sreedhar (2014) observed chronic effect of urea on testicular structure of the black clam. Recently same effects observed in application of ammonium chloride on fish *Clarias batrachus* by Sangeeta, *et al.*, (2020). The above reporting is similar to the present findings.

CONCLUSION:

It could be concluded that *Anabas testudineus* with average weight 30.0± 4.0 g, exposed with fertilizer, urea, 27.5 mg/l, at blood metabolite and histopathological observation were found the liver showed vacuolar degeneration, necrosis, hyperemia, kidneys displayed glomerulonephritis, vacuolar degenerative changes in the tubular epithelium and slight congestion, intestine showed necrosis and desquamation in epithelium cells at the apex of the villi, sperm showed very significant histopathological changes, condensation of spermatogonic cells as well as inflammation of cells, contraction and vacuolation of tubules and while ovary showed degeneration of epithelial cells causes vacuolation, breakdown of germinal vesical. So, it is suggested that more suitable to culture at water fertilizer, urea concentration of < 27.5 mg/l for optimum growth performance. The information will be major role on different levels of responses of organisms with respect to pollutant stress is a necessary pre-requisite for the proper management of fertilizer application in agriculture and aquaculture.

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