



Acute high dose Of L-Lysine amino acid leads nephrotoxicity and hepatotoxicity in fresh water fish *Clarias batrachus*

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Abstract

The present investigation was carried out to find out the effects of high dose of L-lysine on the fish and also an attempt has been made to predict the impact on human health. L-lysine is an essential amino acid which is used in medical practices as a growth promoter of bones especially in infants. Important work on metabolism and functions of amino acids in human health and major diseases has been reported by many workers and evaluated the key role of various amino acids in response to infections. Lysine is not synthesized by body; therefore, it must be taken either by diet or supplementation. Lysine first of all isolated from casein in 1889 and introduced as Lysine hydrochloride in 1955. The recommended dose of lysine is 12mg/kg body weight. Side effects of Lysine in large doses i.e. 10-30gm/day may cause abdominal cramps and diarrhea but renal and hepatic toxicity is not reported in normal person. Amino acids are used to treat end stage renal failure and also in liver failure. Recently lysine is used to treat herpes and as a supplement for diabetic people. In present study the effect of acute high dose 2mg/100gm body weight of L-lysine in fish shows toxic effects on kidney and liver which is an alarming indication towards use of high dose of Lysine in man. In kidney it caused glomerular dilation of the capillaries, glomerular hemorrhages and cloudy swelling of renal tubules. In liver it leads swelling of hepatocytes, vacuolization, fatty degeneration and central vein dilation. These histopathological changes are supported by marked rise in level of blood urea, serum creatinine, blood sugar and serum cholesterol.

Key Words: *Clarias*, *Dilation*, *Hepatocytes*, *Hepatotoxicity*, *L-lysine*, *Nephrotoxicity*

Introduction

Amino acids are basic to life, there are twenty amino acids, of these ten are essential & other ten are non-essential. L-lysine is a basic essential amino acid and not synthesized by body, it is synthesized by plants & bacteria from aspartic acid. Natural sources of Lysine are red meat, chicken, eggs, fish, beans, nuts, soybeans, spirulina etc. It is being used as one of the nutritional supplement in commercially available food products such as Promolan, Hermin, Astymin Forte, Lysiron and many more nutritional forms as tablets and syrups. Lysine supplements are used in medical practices in malnutrition, chronic diseases, renal and liver disorders, chronic gastrointestinal diseases and alcoholism. Lysine is used to treat herpes Venhan and Sanketh (2017). Recently it is used as a new supplement for nutritional support for diabetic people and people at risk of developing diabetes (John, 2018). Role of amino acids in human body related to health and in some major

diseases described by Munro (1982) and evaluated the key role of various individual amino acids in response to infections. Crim and Munro (1977) evaluated protein and amino acids requirement in relation to formula diet for medical purpose. Medicinal uses of L- Lysine were described by Meenu *et al.* (2011). Recently University of Maryland Medical Centre reported L-lysine is essential for human health, but our body cannot synthesize Lysine so we have to get it from food or supplements. Due to medicinal uses Lysine used in daily diet as it lowers cholesterol, helps to absorb calcium and also forms collagen but in a recommended dose. Lee and Kim (2019) worked and reported therapeutic effects of amino acids in liver diseases. The toxicity of different amino acids to vital systems of body worked out by many workers like (Solez, 1983; Malis *et al.*, 1984; Recusen *et al.*, 1985; Asanuma *et al.*, 2006). Amino acids are used to treat toxicity of heavy metals like chromium in fish by Bali and Singh (1994) and Dhami (2012). Some literature is also available on lysine Nephrotoxicity taken with antibiotics like neomycin, gentamycin, streptomycin. The toxicity

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of lysine is least worked out due to its dietary uses however some references are available on its toxicity specially on kidney ,if taken in large doses with impaired kidney function. Steven *et al.* (2007) reported that L-Lysine caused nephritis and impairment in renal proximal tubule. The use of amino acids in treatment of liver cirrhosis, hepatic comma and hepatic encephalopathy is reported by Park *et al.* (2017). The work on acute lysine toxicity carried out is so important because one must be careful to take lysine as a dietary supplement in large doses.

Material and Methods

For this study healthy living freshwater teleost fish *Clarias batrachus* (Linn.) were selected for experimentation. The fish was obtained from local freshwater sources. Animals acclimatized in glass aquaria in laboratory for 10 days before starting the experiment and fed with commercial fish food twice a day. The animals were divided into two groups. Each group of control and experimental consists 10 animals. The experimental group was given intraperitoneal injection of 2 mg/100 gm of body weight/ day for 96 hours to study acute toxicity of L-lysine. The acute toxicity of L-lysine is carried out by LC₅₀ value and during this 50% of animals died due to toxicity. The fish was injected daily in morning after twenty four hours for 96 hours. After 96 hours of acute treatment kidney and liver dissected out and fixed in alcoholic Bouin's fixative for histopathological investigations. Sections of seven micron were cut in rotator microtome. Staining of tissues carried out in delafield's haematoxylin and alcoholic eosin.

Blood samples were also collected from both control and experimental groups. The blood was taken out by severing tail end. Blood collected in a vial containing anticoagulant EDTA. The hematological parameters were determined by standard methods on Photochem-5 colorimeter. Blood urea was estimated by DAM (Diacetylmonoxime) method, blood glucose estimated by "O" Toluidine method, serum creatinine was calculated by Alkaline Picrate Method and serum cholesterol determined by Wybenga and Pileggi Method. The blood parameters data was statistically analysed by calculation of t- test value for difference of means.

Calculation of t-Test for difference of means

't' value was calculated as given under :

$$t = \frac{\bar{x} - \bar{y}}{\sqrt{\frac{\sum(x-\bar{x})^2 + (y-\bar{y})^2}{n_1 + n_2 - 2}}} \times \sqrt{\frac{n_1 \times n_2}{n_1 + n_2}}$$

Where \bar{X} = Mean of the control

\bar{Y} = Mean of the experiment

$(x - \bar{x})^2$ = Sum of squares of deviation from mean in control

$(y - \bar{y})^2$ = Sum of squares of deviation from mean in experiment

n_1 = Sample size of control

n_2 = Sample size of experiment

The histopathology of kidney and liver slides after acute exposure to high dose of L-lysine was observed under Olympus laboratory binocular microscope with high power. Eyepiece camera was used to take the photographs in the department of pathology LLRM Medical College Meerut.

Results and Discussion

The hematological parameters observed for blood urea, serum creatinine, blood sugar and serum cholesterol are given in Table 1 while histopathological changes are shown in fig- 1-3. The findings of renal toxicity are supported by a highly significant statistically increase in blood urea from 5.1 ± 0.2 in control to 8.4 ± 0.45 mg/100 ml after acute exposure and serum creatinine from 0.6 ± 0.041 in control to 3.0 ± 0.72 mg/100 ml after acute treatment. Rise in blood urea and serum creatinine indicates renal dysfunction, these findings are in agreement with Malis *et al.*, (1984), Recusen *et al.* (1985), Asanuma *et al.*, (2006), Samyuktha and Francois (2019). Most of these workers found nephrotoxicity in animals by giving a mixture of many amino acids and they observed increased glomerular filtration rate. In present study the only amino acid lysine was used. The possible mechanism of renal damage seems to be that high dose of dibasic cationic amino acid L-lysine produces a rapid decrease in renal function with tubular obstructions, and later on a decrease in renal blood flow, this depresses the renal function and hence produced acute nephrotoxicity and renal failure that caused death of the fish.



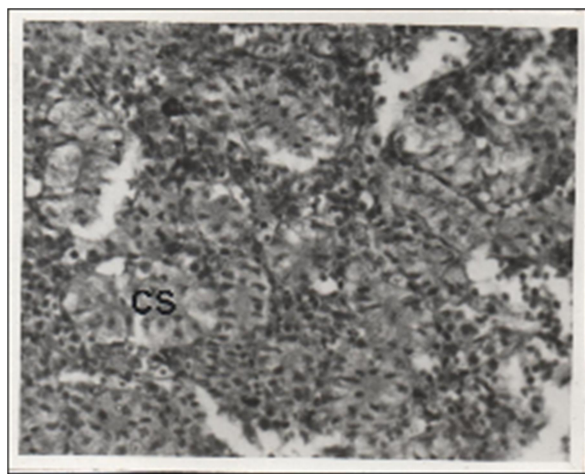


Figure 1. T.S. Kidney(Showing Cloudy Swelling of renal tubules following acute exposure to L- lysine x400) CS=Cloudy Swelling.

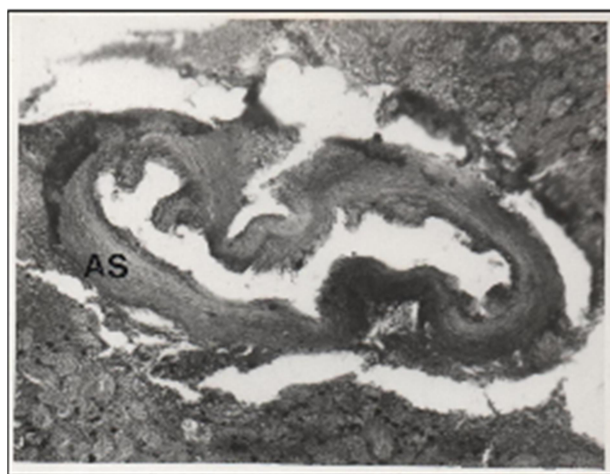


Figure 3. T.S. Kidney(Showing Arteriosclerosis following acute exposure L- lysine x 400) AS= Arteriosclerosis

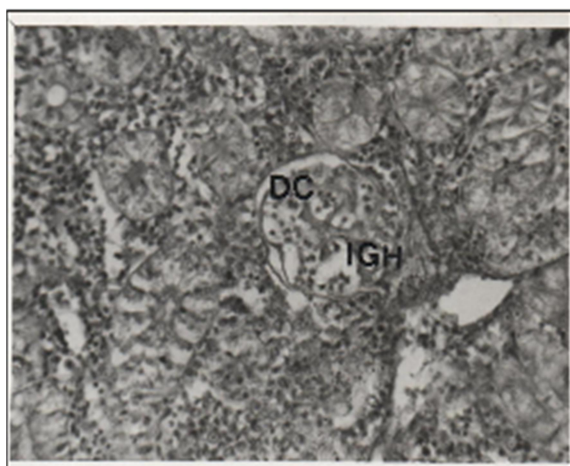


Figure 2. T.S. Kidney(Showing Dilation Glomerulus Capillaries, Interglomerular hemorrhages due to acute exposure to L- lysine x 400) DC= Dilated Capillaries, IGH=Interglomerular Hemorrhages

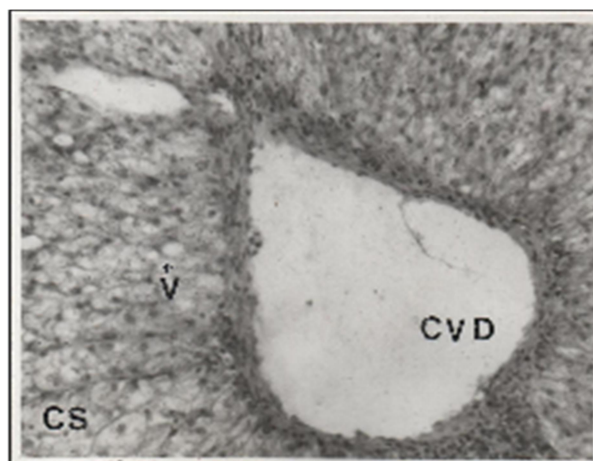


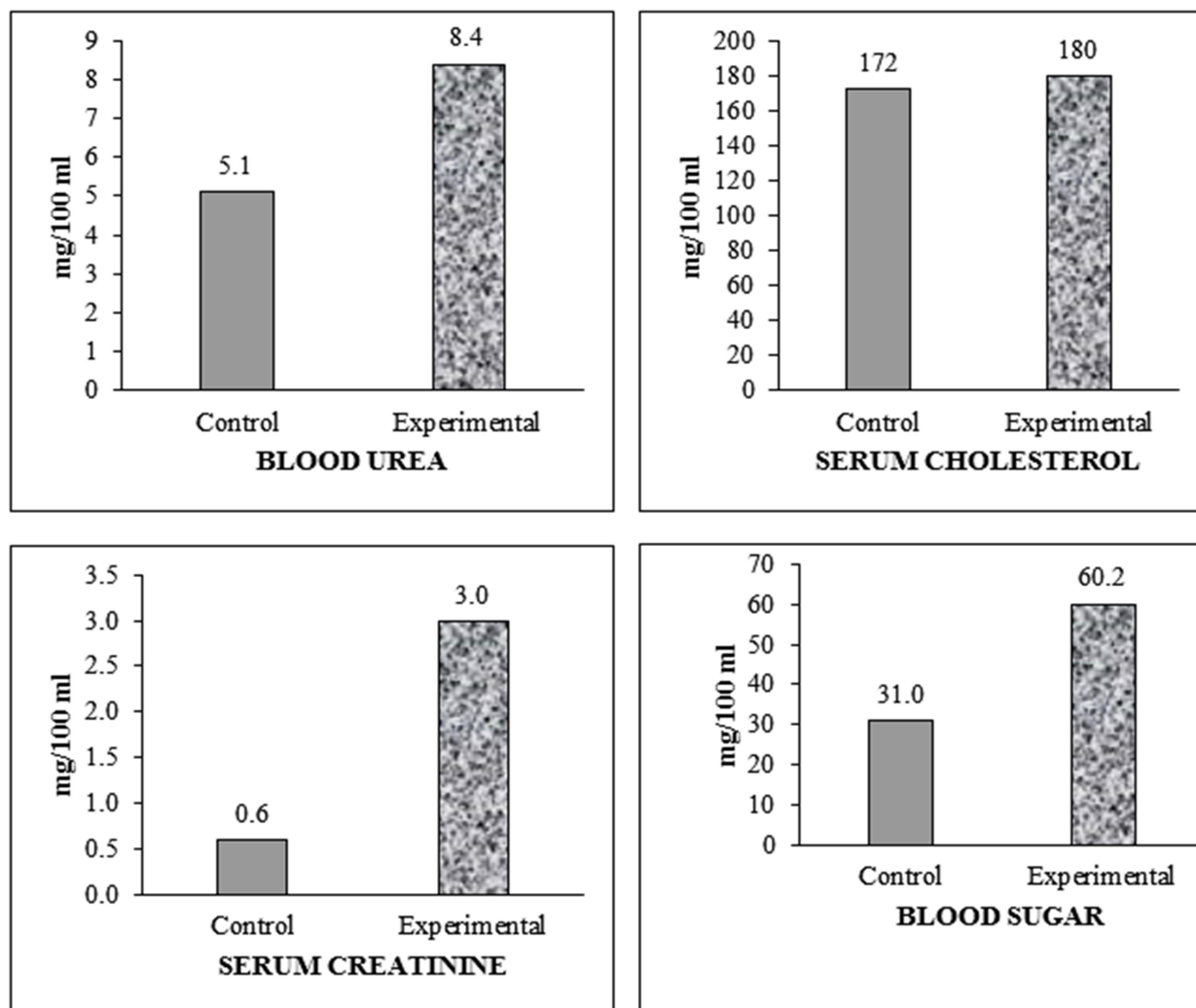
Figure 4. T.S. Liver (Showing Cloudy Swelling , Vacuolization and Central Vein Dilation after acute exposure to L- lysine x400) CS=Cloudy Swelling, CVS=Central Vein Dilation, V=Vacuolization

Arteriosclerosis is a new finding and not found by previous workers in fish and it shows that a high dose of L-lysine may prone to be harmful even in our case because lysine is widely used as a nutritional supplement. However it is known that lysine increases calcium absorption but not reported arteriosclerosis. These studies extend the observation that acute high dose of L-lysine amino acid in fish produced a rapid and permanent decrease in renal function (Steven *et al.*, 2007). It is now reported that a normal intake of Lysine plays an important role in calcium absorption from intestine (Meenu *et al.*, 2011). Present study shows

that arteriosclerosis occurred due to high dose of Lysine because lysine absorbed large amount of calcium from intestine that caused hypercalcaemia. Nephrotoxicity of Lysine may be related to direct tubular toxicity and to tubular obstruction (Malis *et al.*, 1984). These findings of renal toxicity are in agreement with several researchers (Solez, 1983; Malis *et al.*, 1984; Recusen *et al.*, 1985; Asanuma K *et al.*, 2006). The acute exposure to L-lysine in experimental fish caused hepatotoxicity such as cloudy swelling of hepatocytes, vacuolization, central vein dialation and fatty degeneration. These hepatic changes are shown in figure-4. All these

Table 1. Hematological parameters observed for blood urea, serum creatinine, blood sugar and serum cholesterol

SN	Blood Parameter	Control	Experimental	% alteration from control	t - value
1	Blood urea(mg/100 ml)	5.1±0.25	8.4±0.45	64.70%	10.32++
2	Serum creatinine (mg/100ml)	0.6±0.041	3.0±0.72	400%	39.45++
3	Blood sugar(mg/100ml)	31±2.13	60.2±3.14	94.19%	31.62++
4	Serum cholesterol(mg/100ml)	172±9.05	180±7.64	4.65%	2.60

**Figure 5. Bar diagrams of blood parameters after acute exposure to L-Lysine.**

histopathological findings are supported by hematological parameters such as highly significant statistical increase in level of blood sugar from 31 ± 2.13 in control to 60.2 ± 3.14 mg/100 ml after acute exposure to high dose of L-lysine, it shows impaired sugar metabolism. Level of serum cholesterol is not increased significantly which

indicates that lysine does not interfere at a high degree with lipid metabolism even in a very high dose. Therapeutic use of a mixture of amino acids in liver diseases shows their healing and regenerative role. Many workers used Branched Chain Amino Acids (BCCAs) therapy in liver cirrhosis. Clinical trials have suggested that BCCAs

supplementation improves the prognosis of cirrhotic patients and prevented hepatic failure (Muto *et al.*, 2005). Some workers reported increased level of cholesterol in animals when fed on a high lysine dose. These findings of hypercholesterolemia are not in agreement with present study. Hepatotoxicity of lysine in mammals to this extent has not been reported. Essential L-amino acids are used to treat renal disorders by many workers (Denis *et al.*, 2016). The use of amino acids with Keto acids in the treatment of chronic renal failure was reported by Jungers and Chauveau (1988). All these workers applied essential amino acids along with Keto acids to treat the renal failure but no Nephrotoxicity of such supplements reported in chronic and acute treatments. The mixture of essential amino acids with Keto acids also used in treatment of liver failure. Recently the branched- chain amino acid therapy used in liver disorders (Charlton, 2006; Holecek, 2010; Tahira and Khan, 2015). These workers reported that use of these supplements in

end stage liver failure reduced the mortality.

Conclusion

All these findings show that the clinical use of essential amino acids is a new concept to treat the kidney and liver diseases but the toxicity of these supplements further warns their use in high doses. The present studies show that use of L-lysine amino acid in high doses is not safe and its further clinical trials are required to investigate because in this study it is found that lysine is much toxic in acute dose to vital organs like kidney and liver. The present studies show that essential amino acid L-lysine caused nephrotoxicity and hepatotoxicity in experimental fish after acute treatment. In kidney it caused dilation of glomerular capillaries, glomerular haemorrhages and cloudy swelling of renal tubules with vacuolization. Vasodilation, and arteriosclerosis also observed in kidney, all these renal findings show toxicity of L-lysine in acute high dose.

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