Quest Journals
Journal of Medical and Dental Science Research
Volume 2~ Issue 1 (2015) pp: 06-12
ISSN(Online) : 2394-076X ISSN (Print):2394-0751
www.questjournals.org

Research Paper

Acute Toxicity and Biochemical Response of *Clarias batrachus* Exposed To *Adenia cissampeloides* Stem Extract

Ibiam U. A., Egwu C.O., Ezeani N., Edwin N., Okechukwu Ugwu P.C. and Alum E.

Department of Biochemistry Ebonyi State University, Abakaliki, Nigeria.

ABSTRACT:- The study was designed to investigate the phytochemical composition of the *Adenia cissampeloides*, its acute toxicity and biochemical effects in *Clarias batrachus* fish. A total of 144 *Clarias batrachus* juveniles of average weight, 122 g, were randomized into six different groups and exposed to 0.0, 1.25, 2.50, 5.0, 10.0 and 20.0 g/l of the extract, respectively, for 72 hours. Extract concentrations were changed after every 24 hours. For the sub-acute toxicity tests, *Clarias batrachus* juveniles were exposed to 0.0, 0.625, 1.25, 2.5 and 5.0 g/l of *Adenia cissampeloides* extract for 8 hours. Four fish were sampled from each group at I hour interval. Blood samples were collected from incisions made at the thoracic cavity of the fish using syringes. The serum was used for the biochemical assay of AST, ALT, ALP, protein, bilirubin, malondialdehyde (MDA), SOD, GST, and total cholesterol. The phytochemical composition and all biochemical parameters were determined using standard methods. The results showed that *Adenia cissampeloides* stem-bark has high contents of tannin and flavonoids, alkaloids and glycosides; the 24, 48 and 72 hr LC_{50} were 5.0, 2.5 and 2.5 g/l, respectively. There was a significant increase ($p<0.05$) in the AST activity within the first 1 hour of exposure of the fish to the extract in a time and concentration-dependent manner. There was statistically significant increase ($p<0.05$) in the concentrations of total protein, unconjugated bilirubin, malondialdehyde and cholesterol. Though there was increases in the activities of ALT, ALP and SOD, these were not significant ($P>0.05$). But the GST activity showed a significant reduction ($p<0.05$). The results show that *Adenia cissampeloides* is toxic to *C. batrachus*.

Keywords:- *Adenia cissampeloides, Clarias batrachus,* lethal concentration(LC_{50}), acute toxicity and biochemical responses.

I. INTRODUCTION

If swallowed or absorbed into the body. They are used to stupefy or kill fish without making them toxic to humans. Some synthetic chemicals and toxic principles contained in certain plants are used ad fish poisons. There are usually used in small river or pools with slow moving water.

Fish poisons are substances that cause death or harm to fish. They are used mostly in Tropical America, Australasia and Tropical Africa to harvest fish from streams and rivers. It has been reported that they exert their toxicity by **affecting a number of cellular processes in the fish, for example, they inhibit Na^+-K^+ ATPase, interfere with oxidative capacity and glutathione metabolism of major organs and nerves, and cause other toxic effects (Ray, 1991, Ratra, *et al.*, 2001).** Many plants species have been used as fish poison. Examples are: *Psychothra microphylla* (Orji *et al*., 2014), *Monkshood, Alphitonia, Quercus* and *Olax* (Rashtra, 2006). The plants are known to contain metabolites that stupefy the fish, make them conscious and therefore easy to catch (Van-Andel, 2000). Notable among such active principles are rotenones and saponines (Bearez, 1998). Some plants which also liberate cyanide in water are also used as fish poison, as well as plants which contain high levels of ichthyothereol, triterpene and other ichthyotoxins (……).

The ancient practice of poisoning fish was an important method of securing food and has continued to flourish in many cultures today. People consume fish produced by this method in almost every part of the world. In Ebonyi State and possibly many other parts of Nigeria, the stem extract of *Adenia cissampeloides* is used as fish poison and such fish are widely consumed in the state.

Adenia cissampeloides is a robust semi-woody climber of the family *passyfloraceae*. It produces gum resin which contains modeccin, which is toxic to humans (Hutchings *et al.*, 1996).

The plant is economically important as it is used for various purposes in different countries and localities. The stem is used as fish poison when macerated in water and as emetic for biliousness (Brandt and

Muller, 1995). Fresh leaves of *Adenia cissampeloides* are used as vegetables in Ngbo, Ebonyi State; in Ivory Coast, the leaf extract is rubbed on the breast after child birth to promote lactation (Irvine, 1961).

To the best of our knowledge, no research has been carried out on the toxicity of this plant and its biochemical effects to *Clarias batrachus* . This was the primary goal of this research.

II. MATERIALS AND METHODS

The major equipments were Spectrophotometer (Spec 21D), centrifuge (MSE minor-35),deep freezer (Philip), incubator (XM020), and weighing balance (ICD2000).

Collection of plant and Fish Samples

Adenia cissampeloides plant was obtained from a river side located at Enyibuchiri Okposhi-Eshi Ngbo, Ohaukwu L.G.A, Ebonyi State and identified by Prof J.C. Okafor of tree crop and tropical ecology consultancy (TEC), No 7 Dona drive, off Iheala street, Independence Layout, Enugu, Nigeria. *C. batrachus* (air breathing catfish) was purchased from Fishery Department, Ebonyi State University and transported to the Department of Biochemistry, Ebonyi State University, where the analyses were carried out.

Preparation of plant extract and exposure of Fish to Toxicants

The aqueous extract of *Adenia cissampeloides stem* was prepared using the methods of Akinside and Olukoya (1995) and Akinyemi *et al.,* (2000). About 100 g of the stem was soaked in 100 ml of distilled water for 12 hours and then filtered using Whatman filter paper.

Screening of Phytochemicals

The phytochemical screening for tannins, saponin, flavonoids, alkaloids, cardiac glycosides and steroids were determined using the methods described by Harbone (1973), Trease and Evans 1989) and Sofowara (1993).

Acute Toxicity Test

A total of 144 *Clarias batrachus* juveniles of average weight, 122 g, were randomized into six different groups and exposed to 0.0, 1.25, 2.50, 5.0, 10.0 and 20.0 g/l of the extract, respectively, in a semi-static tank for 72 hours. Each group was monitored at 24 h interval for 72 hours and their lethal concentrations (LC_{50}) determined. Extract concentrations were changed after every 24 hours.

Sub-acute Toxicity Test and Biochemical Analysis of Samples

For the sub-acute toxicity tests, a total of 160 *Clarias batrachus* juveniles were exposed to 0.0, 0.625, 1.25, 2.5 and 5.0 g/l of *Adenia cissampeloides* extract for 8 hours. Four fish were sampled from each group at I hour interval. Blood samples were collected from incisions made at the thoracic cavity of the fish using syringes. The serum was used for the biochemical assay of AST, ALT, ALP, protein, bilirubin, malondialdehyde (MDA), SOD, GST, and total cholesterol. The biochemical parameters were determined using standard methods. The AST, ALT and ALP were measured by colorimetric method (Reitman and Frankel (1957), Schmidt and Schmid(1963). The total serum protein was measured by using the standard Biuret method (Hartree (1972) Lowry (1951). Total and direct bilirubin was determined by method of Jendrassik and Grof (1938). Total cholesterol was measured using colorimetric method of Zak (1957).

Statistical Analysis

All the results obtained were expressed as mean ± S.D of 5 rats in each group .All the tested parameters were subjected to statistical analysis using ANOVA. Differences between means were regarded significant at $P<0.O5$

III. RESULTS AND DISCUSSIONS

Phytochemical analysis

The results of the phytochemical analysis and acute toxicity tests are presented in Tables 1 and 2, respectively. It showed that the *Adenia cissampeloides* stem has high content of tannin, flavonoids, alkaloids, glycosides and saponin. The results are true reflection of the phytochemical constituents of the plant and genus generally as have been reported by Bearez (1998) and Prance and Balick (1990). The results showed that *Adenia cissampeloides* stem contain principles that are poisonous to fish. The active principles of fish poison plants include rotenone which interferes with respiration by inhibiting the protective mechanism of GST in minimizing the toxic potential of oxygen intermediates. Saponin has asphyxiation action while cardiac glycosides affect the central nervous system and nerve mechanisms of the heart (Prance and Balick, 1990). Alkaloids have neurologic

effects; they inhibit Na^+-K^+ ATPase, DNA polymerase, cytochrome P-450 system and cause damage to the liver cells. Tannins cause enzyme inhibition, substrate and metal ion deprivation and other slow acting effects (Hagerman and Klucher, 1986).

Acute Toxicity

The results of the acute toxicity tests showed that the plant was toxic to *C. batractus* species; the effect was time and concentration-dependent. The 24, 48 and 72 hr LC_{50} values calculated using Probit analysis were 5.0, 2.5 and 2.5 g/l, respectively.

Biochemical Parameters

Exposure of animals to certain levels of toxicants may alter the mechanisms required for maintaining a healthy physiological state. In view of this, there has been increasing interest in examining the physiological and biochemical stress responses of aquatic vertebrates to toxicants so as to protect aquatic life. Majority of biochemical parameters are routinely used to assess health status and aid in the diagnosis of diseases in man and animals.. In this study, the biochemical responses of *C. batrachus* exposed *Adenia cissampeloides* plant stem extract were determined. A dose and time dependent increase was observed for AST in this study (Figure 1). Within the first one hour of exposure, the activity of AST increased from 17.52 µ/l in the control to 46.25 µ/l in the highest concentration of 5.0g/l extract and 76.0 µ/l within eight hours. This could be attributed to the toxic effect of the extract on the liver of the exposed fish. A similar trend of increase was observed for ALT (Figure 2), but not significant ($p>0.05$). However, the AST/ALT ratio of 1:5 showed significant damage to the liver, heart and muscle cells, which may have caused a release of membrane-bound AST (Younossi, 1998)

An increase in the activity of ALP was also observed in the study (Figure 3). The ALP rose from 4.83µ/l in the control to 42.94µ/l in one hour to 66.46 µ/l within eight hours at the highest exposure concentration of 5.0g/l of the extract. An elevated ALP is usually associated with liver or bone disease (Price, 1993).

A dose and time dependent increase in TP concentration was observed in the study (Figure 4). Increase in total protein usually high albumin is almost always caused by dehydration (Gaull, 1984). The increase in TP implied that dehydration occurred in the fish exposed to *Adenia cissampeloides* stem extract.

In the same the vein, the concentration of unconjugated bilirubin increased in a time and dose-dependent manner(Figure 5). This suggests that haemoglobin was being destroyed or the liver was not actively treating the haemoglobin it received. This agrees with the result of elevated AST activity, increase in unconjugaated unconjugated bilirubin up to 0.473 mg/dl coupled with AST/ALT ratio 0f 1:5 indicate liver damage (Nyblom *et al.*, 2006). There was a small increase in MDA (Figure 6).

There was a significant reduction in the SOD activity ($P<0.05$) in a time and dose-dependent manner (Figure 7). Reduction in the ability of SOD to inhibit free radical generation suggests autoxidation which might have led to oxidative stress.

The result further revealed that the activity of GST significantly ($p<0.05$) decreased as the concentration of the extract and the time of exposure increased(Figure 8). Edwards et al. (2000) reported that, glutathione transferase play an important role in the detoxification of ROS in the cells and protecting the lipids from peroxidation. This implies that the ability to detoxify toxins is reduced.

Total cholesterol increased significantly ($p<0.0.05$) in time and dose(Figure 9). This rise in cholesterol is an indication and probably suggests a general increase in lipid mobilization. Hypercholestrolemia observed may be due to impairment of liver and inhibition of enzymes which convert cholesterol into bile acid (Murray,1991)

IV. CONCLUSION

The results in this study on the acute toxicity showed that the 24, 48 and 72 hr LC50 were 5.0, 2.5 and 2.5 g/l, respectively.

The acute toxicity could be associated to the presence of fish poison principles in *A. cissampeloides* stem extract.

REFERENCES

[1]. Akinside, K. A. and Olukoye, D. K. (1995). Vibriocidal Activities of some local herbs. *Journal of Disease Research*,**13**:127-129.
[2]. Akinyemi, K. O., Bayagbon, C. and Coker, A. O. (2000). Antibacterial screening of five indigenous Nigerian Plants against *S. typhi* and *S.paratyphi. Journal of Nigerian Infection control association,* **3**(1): 30-33.
[3]. Bearez, P. (1998). Focus: First Archeological indication of fishing by poisoning in a sea environment by Engoroy population at Salango (Manabi Ecuador). *Journal of Archeological science,* **25**:943-948.
[4]. Brandt, H. D. and Muller, G.J. (1995). Traditional medicines and acute poisoning. *Journal of continuing medical Education, 13*: 1053-1060.
[5]. Hagerman, A. E. and Klucher, K. M. (1986). Tannin-protein interaction in plants flavonoids in biology and medicine: biochemical, pharmacological and structure-activity relationship. Ed. Cody, V., Middletor, E Jr., Harbone J-Alan R. Lis, New York, Pp 67-76.

[6]. Harbone, J. B. (1973). Phytochemical methods. London Chapman and Hall Ltd. Pp49-188.
[7]. Hutchings, A., Scott, A. H., Lewis, G. and Cuningham, A. B. (1996). Zulu medicinal plants-an inventory . Scottville. University of Natal press. Pp 450.
[8]. Irvine, F. R. (1961). Woody plant of Ghana, with special reference to their uses. London, Oxford University press.
[9]. Prance, A. and Balick, J. (1990). The occurrence of piscides and supefactants in the plant kingdom. *Advances in economic Botany*, **8**:256-273.
[10]. Rashtra, V. (2006). Floristic plants of the world. Amazon. Sarup & Sons. Pp 949.
[11]. Ratra, G. S.,Kamita, S.G. and Casida, G. E. (2001). Role of human GABA receptor bta3 summit in insecticide toxicity. *Toxicology, Applied pharmacology*, **172**: 233-240.
[12]. Ray, D. E.(1991). Pesticides derived from plants and other organism. In handbook of pesticide toxicology. Hayes, W.J. Jr, and Laws , E. R. Jr Eds. New York academic press.
[13]. Sofowara, A.(1993). Medicinal plants and traditional medicine in Africa. Ibadan Nigeria. Spectrum Book Ltd Trease, G. E. and Evans, W.C. (1989). Pharmacognosy. 11th edition. Brailliar Tridelcan. Macmillian publishers.
[14]. Van-Andel, T.(2000).the diverse uses of fish-poison plants in North West Guyab. *Economic Botany*, **54**: 500-502.
[15]. Akinside, K. A. and Olukoye, D. K. (1995).Vibriocidal Activities of some local herbs. *Journal of Disease Research*,**13**:127-129.
[16]. Akinyemi, K. O., Bayagbon, C. and Coker, A. O. (2000). Antibacterial screening of five indigenous Nigerian Plants against *S. typhi* and *S.paratyphi. Journal of Nigerian Infection control association*, **3**(1): 30-33.
[17]. Bearez, P. (1998). Focus: First Archeological indication of fishing by poisoning in a sea environment by Engoroy population at Salango (Manabi Ecuador). *Journal of Archeological science*, **25**:943-948.
[18]. Brandt, H. D. and Muller, G.J. (1995). Traditional medicines and acute poisoning. *Journal of continuing medical Education*,**13**: 1053-1060.
[19]. Edwards R, Dixon DP, Walbot V (2000). Plant glutathione Stransferases: enzymes with multiple functions in sickness and in health. Trends Plant Sci. 5: 193-198.
[20]. Gaull, H., Wright, C. E. and Gaul, G. E. (1984). Protective effect of taurine, zinc and tocopherol on retinol-induced damage in human lymphoblastoid cells. *Journal of Nutrition*,**114**(12): 2256-61.
[21]. Harbone, J. B. (1973). Phytochemical methods. London Chapman and Hall Ltd. Pp49-188.
[22]. Hartree, E.F.(1972) *Analytical Biochemistry*, 48, 422 – 427.
[23]. Hutchings, A., Scott, A. H., Lewis, G. and Cuningham, A. B. (1996). Zulu medicinal plants-an inventory . Scottville. University of Natal press. Pp 450.
[24]. Irvine, F. R. (1961). Woody plant of Ghana, with special reference to their uses. London, Oxford University press.
[25]. Jendvassik, L. and Grof, P. (1938). Bilirubin assay in liver disease. *Biochemistry*, **297**:81 Lowry, O.H., Rosenberg, N.J., Farr, A.L. and Randall, R.J.(1951). *Journal of Biological Chemistry*, 164, 321 – 329.
[26]. Murray R.K. *Harpers* Biochemistry 22nd edn, Prentice Hall International Inc. **1991,** Pp678
[27]. Nyblom, H., Bjornsson, E., Simren, M., Aldenborg, F., Almer,S. and Olsson, R. (2006). The AST/ALT ratio as an indicator of cirrhosis in patients with PBC. *Liver International*, **26**(7): 840-845.
[28]. Price, C. P. (993). Multiple forms of human serum alkaline phosphatase: Detection and Quantification. *Annals of clinical biochemistry*, **30**: 355-372.
[29]. Rashtra, V. (2006). Floristic plants of the world. Amazon. Sarup & Sons. Pp 949.
[30]. Ratra, G. S.,Kamita, S.G. and Casida, G. E. (2001). Role of human GABA receptor bta3 summit in insecticide toxicity. *Toxicology ,Applied pharmacology*, **172**: 233-240.
[31]. Ray, D. E.(1991). Pesticides derived from plants and other organism. In handbook of pesticide toxicology. Hayes, W.J. Jr, and Laws , E. R. Jr Eds. New York academic press.
[32]. Reitman, S. and Frankel, S. (1957). Assay of transaminase. *American Journal of clinical pathology*, **28**: 56-63.
[33]. Schmidt, E. and Schmidt, F. W. (1963).assay of transaminase. Enzymological, *biological and Clinical Journal*, **3**:1.
[34]. Smith, P.K. (1985). Determination of total protein. *Analytical Biochemistry*, **150**: 76-85.
[35]. Sofowara, A.(1993). Medicinal plants and traditional medicine in Africa. Ibadan Nigeria. Spectrum Book Ltd Trease, G. E. and Evans, W.C. (1989). Pharmacognosy. 11th edition. Brailliar Tridelcan. Macmillian publishers.
[36]. Van-Andel, T.(2000).the diverse uses of fish-poison plants in North West Guyab. *Economic Botany*, **54**: 500-502.
[37]. Younossi, Z. M. (1998)evaluating asymptomatic patterns with mildly elevated liver enzymes. *Cleveland Clinic Journal of medicine*, **65**:150-158.
[38]. Zak, B. (1957). Determination of Total cholesterol. *American Journal of clinical pathology*, **27**: 583.

Table 1: Phytochemicals present in *Adenia cissampeloides*

Phytochemicals	Observations	Inference
Tannin	Greenish precipitate	+++
Saponin	Stable form, no emulsion	+
Flavonoids (rotenone)	Brick-red colour	+++
Alkaloids	Turbid and dark precipitate	++
Glycosides	Brick-red precipitate	++
Steroids	No reaction	-

Table 2: Lethal concentration of the *Adenia cissampeloides* stem extract on *C. batrachus*

	No of dead fish exposed to extract							
Time(hour)	Total fish	0.0 g/l	1.25 g/l	2.5 g/l	5.0 g/l	10.0 g/l	20.0 g/l	LC_{50} (g/l)
24	48	0	0	2	4*	8	8	5.0
48	48	0	1	4*	6	8	8	2.5
72	48	0	2	4*	6	8	8	2.5

* indicates the death of half of the population of fish exposed to a given extract concentration significant ($p>0.05$), (Table 3).

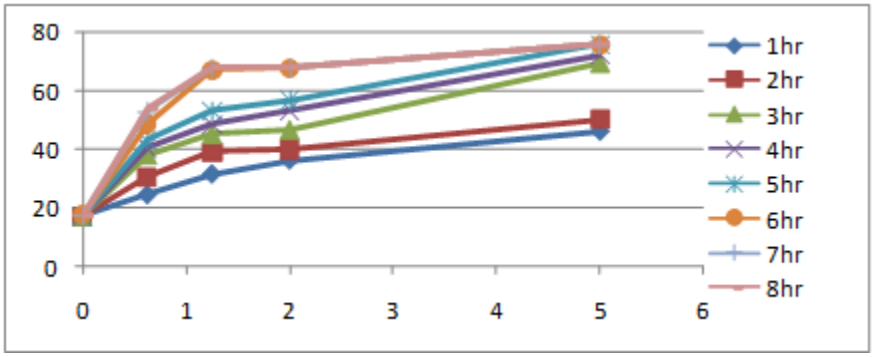

Figure 1: Effects of *Adenia cissampeloides* stem extract on AST activity in *C. batrachus* within eight hours of exposure

The activity increased significantly ($p<0.05$) with increase in extract concentration and time

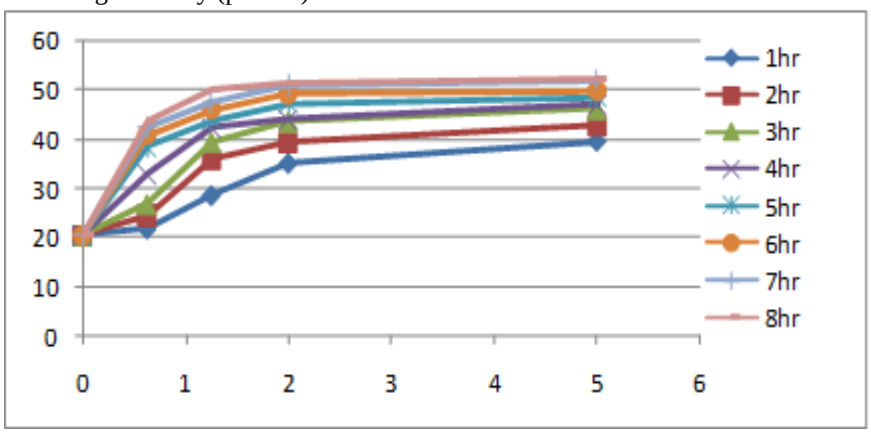

Figure 2: Effects of *Adenia cissampeloides* stem extract on ALT activity in *C. batrachus* within eight hours of exposure

The activity increased non-significantly ($p>0.05$).

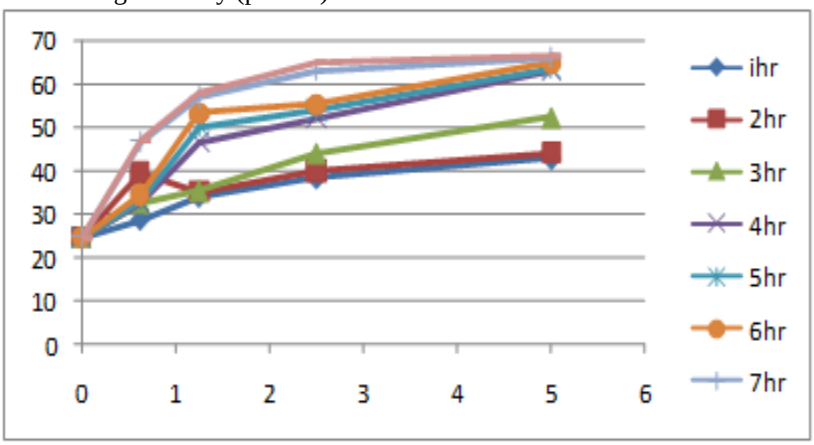

Figure 3: Effects of *Adenia cissampeloides* stem extract on ALP activity in *C. batrachus* within eight hours of exposure

The activity of ALP increased non-significantly.

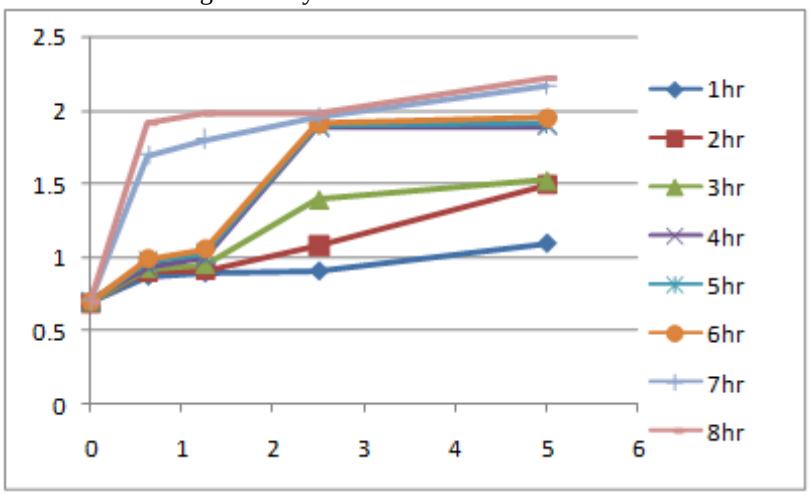

Figure 4: Effects of *Adenia cissampeloides* stem extract on Total protein (TP) Concentration in *C. batrachus* within eight hours of exposure

The TP increased significantly ($p<0.05$) with time and concentration

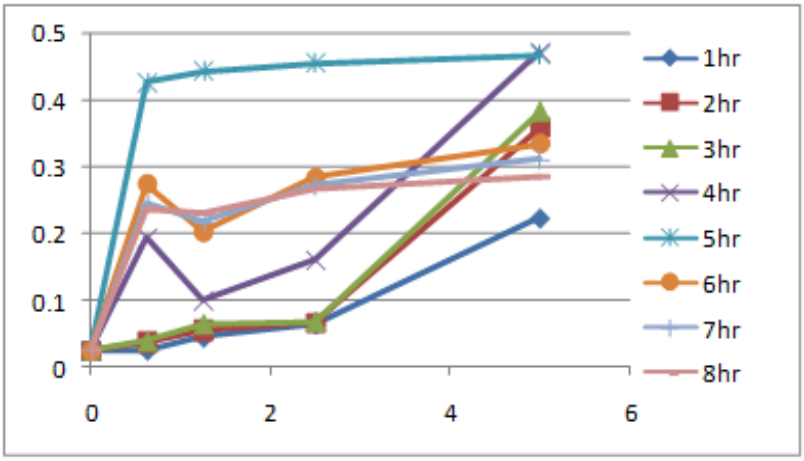

Figure 5: Effects of *Adenia cissampeloides* stem extract on Unconjugated bilirubin(UB) Concentration in *C. batrachus* within eight hours of exposure

The unconjugated bilirubin concentration (mg/dl) increased significantly ($p<0.05$)

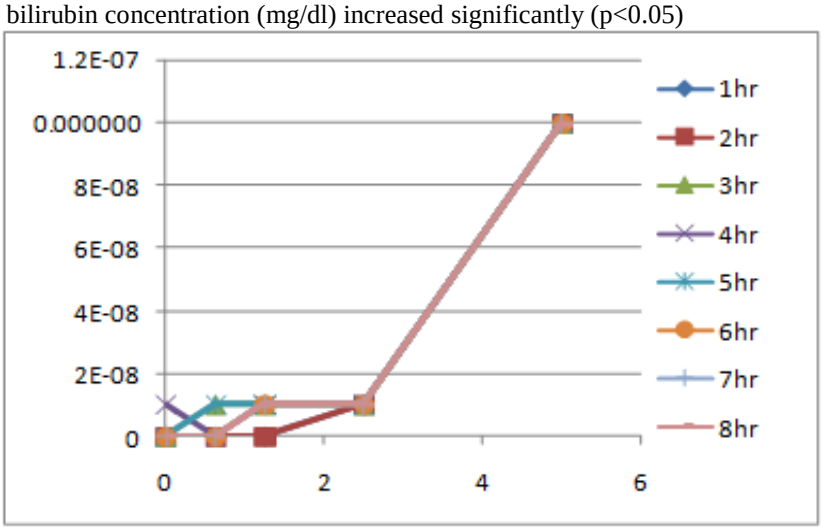

Figure 6: Effects of *Adenia cis*sampeloides stem extract on MDA concentration in *C. batrachus* within eight hours of exposure

The MDA concentration (mg/dl) increased significantly ($p<0.05$

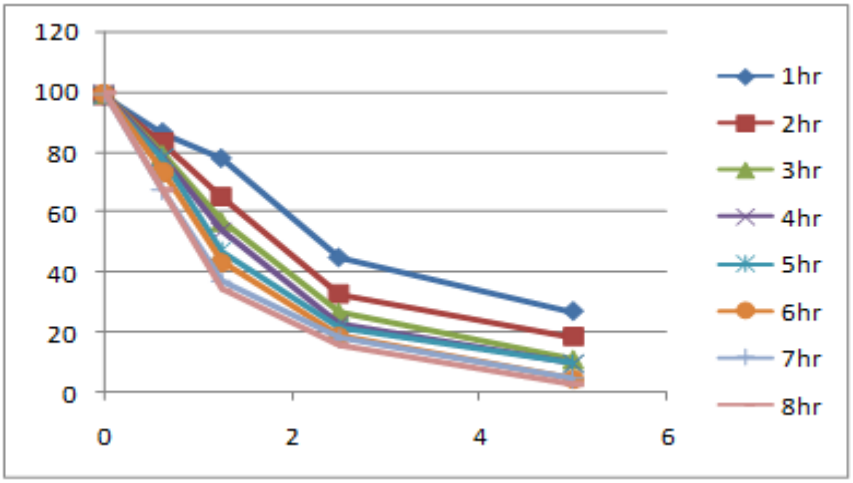

Figure 7: Effects of *Adenia cissampeloides* stem extract on SOD activity in *C. batrachus* within eight hours of exposure

Non-significant decrease in SOD activity.

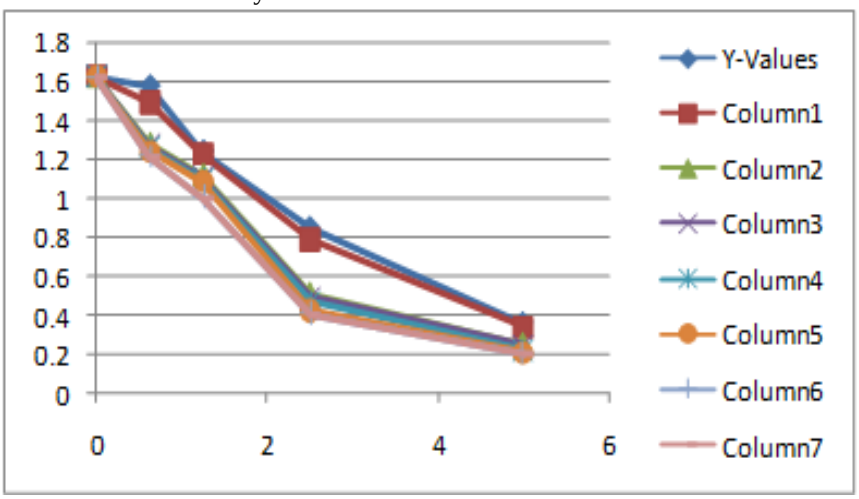

Figure 8: Effects of *Adenia cissampeloides* stem extract on GST activity in *C. batrachus* within eight hours of exposure

GST decrease was statistically significant ($p<0.05$)

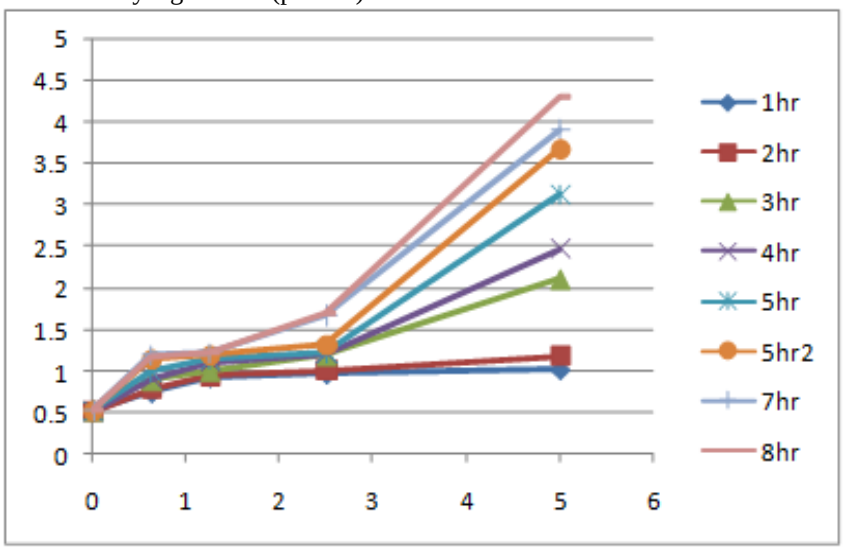

Figure 9: Effects of *Adenia cissampeloide*s stem extract on Total cholesterol concentration in *C. batrachus* within eight hours of exposure