Toxicity and Effect of *Carica Papaya* Seed Aqueous Extract on Liver Biomarkers of *Clarias Gariepinus*

Joseph E Eyo
Department of Zoology and Environmental Biology, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria Email: joseph.eyo@unn.edu.ng
Chidinma A Levi
Department of Zoology and Environmental Biology, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria Email: chidinma.levi@unn.edu.ng
Chinweike N Asogwa
Department of Zoology and Environmental Biology, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria Email: normi4u@yahoo.com chinweike.asogwa@unn.edu.ng
Elijah C Odii
Department of Zoology and Environmental Biology, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria Email: elijahrelateswithjesus@yahoo.com
Christian O Chukwuka
Department of Zoology and Environmental Biology, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria Email: onyichrismac@yahoo.com
Njoku Ivoke
Department of Zoology and Environmental Biology, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria Email: njoku.ivoke@unn.edu.ng
Uwakwe S Onoja
Department of Home Sciences and Nutrition, Faculty of Agriculture, University of Nigeria, Nsukka, Enugu State, Nigeria. Email: uwakwe.onoja@unn.edu.ng
Christopher C Onyeke
Department of Plant Science and Biotechnology, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria. Email: christopher.onyeke@unn.edu.ng

ABSTRACT

The mesocarp of pawpaw fruits (*Carica papaya*) is a delicacy in the tropics. However, the seeds contain toxic substances such as carpine, papain, etc. The first phase of the present study determines the acute toxicity of *C .papaya* seed extract to *Clarias gariepinus* juveniles using static bioassay. Sixty juveniles with mean weight 3.86 ± 1.18g and mean length 9.5 ± 1.52cm were exposed to triplicate concentrations of 0, 150, 225 and 300mg/l in twelve 15L plastic tanks, with each replicate having 5 fish. There were 93.33, 66.67, 46.67 and 0% cumulative mortalities corresponding to 300, 225, 150 and 0 mg/l of *C. papaya* seed aqueous extract within the of hours of exposure. The 24, 48, 72 and 96 h LC_{50} values were 1200.78, 426.67, 191.76 and 163.02 mg/l of *C. papaya* seed aqueous extract, respectively. These showed that the effects were dose and time dependent. The second phase determined the effect of sub-lethal concentrations of the test substance (0, 50, 75 and 100mg/l) in a renewal bioassay system on the liver biomarkers. Significant dose and time dependent changes ($p \leq 0.05$) in acid phosphatase (ACP), aspartate transferase (AST) and alanine transferase (ALT) activities were noticed. The liver ACP levels were significantly higher ($p \leq 0.05$) at 72 and 144h than at 0h. Both the AST and ALT levels were significantly higher ($p \leq 0.05$) at 72h when compared with 0h and 144h. The behavioural responses by *Clarias gariepinus* in this test were; erratic movement, air gulping, loss of reflex and skin discoloration. The maximum admissible toxicant concentrations ranged from 1.91 to 2.30 log toxicant concentration (at 95% confidence limit). The results obtained showed that concentrations of pawpaw seed extract in excess of 163.02 mg/l can be potentially harmful to *Clarias gariepinus* juveniles.

Keywords- *Carica papaya*, seed aqueous extract, toxicity, mortality, liver enzymes, *Clarias gariepinus*

1. INTRODUCTION

Pawpaw (*Carica papaya*) is a common fruit available throughout the year in the tropics. The fruits, leaves, seeds and latex are used as a cure for many tropical diseases hence the common name “medicine tree” or “melon of health” [1,2]. The major active ingredients (carpine, chymopapain, papain, bactericidal aglycone, benzyl isothiocyanate, aglycoside, sinigrin, the enzyme myrosin and carpasemine) are present in the seeds [1-3]. The fleshy part of the fruits (mesocarp) is a delicacy and nutrient-rich drinks of high demand are produced from them. However, some of the active substances (e.g. carpine and papain) from pawpaw are toxic [2]. Carpine are present in traces in the black seeds of *C. papaya*. In large quantities, it is used to lower the pulse rate and depress the nervous system.

RECENT SCIENCE Publishing Research

© RECENT SCIENCE PUBLICATIONS ARCHIVES| August 2013|$25.00 | 27702685 |
This article is authorized for use only by Recent Science Journal Authors, Subscribers and Partnering Institutions

Externally, the latex is an irritant, dermatogenic and a vescicant. Internally, it causes severe gastritis. Some people are allergic to the pollen, the fruit and the latex. Papain can induce asthma and rhinitis. The acid fresh latex can cause severe conjunctivitis and vesication. Carpine and papain also have anti-fertility properties and thus can be used in birth control [4]. *Clarias spp* are mostly freshwater fishes which are distributed throughout the African and Asian lakes, swamps and rivers. *Clarias*, however, can be obtained throughout the year in Nigerian rivers and are anadromous. The fish is in high demand because of it rich flesh and good taste. To meet the ever increasing demand, *C. gariepinus* is the fish of choice in Nigerian aquaculture. The aim of this study was to find out the toxicity effects of *C. papaya* seed extract on C. gariepinus by assessing its effect on mortality, liver enzymes levels and behaviour of *C. gariepinus*.

2 MATERIALS AND METHODS

2.1 Experimental Fish

Ninety six *Clarias gariepinus* juveniles with mean length 11.00 ± 3.00 cm and mean weight 6.0 ± 1.5g used in this study were obtained from Agricultural Holdings, Nsukka. The fish were transported to the Fisheries and Hydrobiology Wet Laboratory, Department of Zoology and Environmental Biology, University of Nigeria, Nsukka and acclimatized for two weeks. During the acclimatization, catfish were fed regularly (twice a day) with the Copens fish feed containing 35% crude protein. The water was aerated continuously using aquarium aerators.

2.2 Carica Papaya Seed Aqueous Extract

Twenty five (25) large ripe pawpaw fruits were harvested from the Agriculture Farm, University of Nigeria, Nsukka, Enugu State, Nigeria. The mature seeds were recovered from the fruit, washed and sun dried to constant weight. 500g of the dry seeds was ground into powder using Corona grinder (China) and the resulting powder was soaked in 10 litres of distilled water. The mixture was allowed to stand for 24 hours with intermittent shaking. The mixture was filtered using Whatman filter paper (grade 1: 11 µm) and the filtrate dried into powder using a rotary evaporator (Stuart, model RE-300, UK) and stored in seal vials under refrigeration pending use. The aqueous extract was serially diluted in distilled water to appropriate concentration before administration.

2.3 Acute Toxicity Test

Sixty catfish juveniles with mean weight 3.86 ± 1.18g and mean length 9.5 ± 1.52cm were used for this experiment. The acute toxicity test was conducted to determine the level of toxicity of pawpaw seed aqueous extract. *C. gariepinus* juveniles were batch-weighed and distributed randomly to twelve (15 litres) plastic tanks. Each container was covered with nylon mesh tied firmly with rubber strap to prevent the fish from jumping out. Each treatment group were dosed with 0 (control), 150, 225 and 300 mg/l of *C. papaya* seed aqueous extract [5] and replicated thrice with each replicate containing five fish. The toxicity testing was done using static bioassay whereby there was no aeration, no water change nor feeding throughout the test period [5]. The mortality and behavioural changes of the catfish during the acute toxicity test was monitored for four days and the 96 hour-LC50 determined graphically using probit transformation. The inability of fish to respond to external stimuli was used as an index of death. The temperature, pH, DO and total hardness were 27.0 ± 2.6˚C, 7.50 ± 1.02mg/l, 6.7 ± 0.52mg/l and 110 ± 2.28mg/l equivalent of $CaCO_3$, respectively during the study.

2.4 Sub-Lethal Toxicity Test

Thirty six juveniles of 3.86 ± 1.18g and 9.5 ± 1.517cm were used for this experiment. Each treatment group was replicated thrice with each replicate containing three fish. The pawpaw seed extract was administered at sub-lethal concentrations (1/3 of the LC_{50}) of 0 (control), 50, 75 and 100mg/l in a renewal bioassay system [6]. The water and the tested compound were changed daily without reducing or changing the toxicant concentration. The liver from different treatments groups were assayed for enzyme activities at 0, 72 and 144h. The fish were dissected and their various livers collected. The liver enzymes studied were acid phosphatase (ACP) [7], aspartate aminotransferase (AST) and alanine aminotransferase (ALT) [8]. Apart from monitoring and recording fish mortality, behavioural and dermatological changes such as: erratic swimming, air gulping, loss of reflex, skin discoloration and haemorrhage were monitored.

2.5 Statistical Analysis

Mean values were analysed for significant differences ($p \leq 0.05$) using the analysis of variance (ANOVA). Differences between means were partitioned using the Duncan new multiple range test. The statistical package for social sciences (SPSS), version 17, was used for all analysis. The probit value was determined from the probit model developed by Finney [9].

3. RESULTS

3.1 Behavioural Responses

The catfish juveniles showed behavioural responses to *C. papaya* seed aqueous extract. The behavioural responses were both extract concentration and time dependent with 0mg/l inducing lesser behavioural responses than 300mg/l for both acute and sub-lethal toxicity phases and 24h and 48h producing lesser behavioural changes than 96h and 168h for the acute and sub-lethal toxicity phases, respectively. The observed behavioural responses were loss of reflex, air gulping, erratic swimming, discoloration of skin and haemorrhage (Table 1).

3.2 Mortality

Percentage mortality at 24 h increased with increase in toxicant concentration. Catfish juveniles exposed to 150, 225 and 300mg/l had 6.67, 13.33 and 20.00% mortalities, respectively (Table 2). The 24 h LC_{50} at 95% confidence

© RECENT SCIENCE PUBLICATIONS ARCHIVES| August 2013|$25.00 | 27702685 |
This article is authorized for use only by Recent Science Journal Authors, Subscribers and Partnering Institutions

limit was estimated as 1200.78mg/l (Fig. 1). The percentage mortality at 48h increased with the toxicant concentration. Catfish juveniles exposed to 150, 225 and 300mg/l had 13.33, 13.33 and 20.00% mortalities, respectively (Table 2). The 48h LC_{50} at 95% confidence limit for toxicant concentration was estimated as 426.67mg/l (Fig. 2). The percentage mortality at 72h increased with the toxicant concentration. Catfish juveniles exposed to 150, 225 and 300mg/l had 20.00, 26.67 and 33.33% mortalities, respectively (Table 2). The 72h LC_{50} at 95% confidence limit for toxicant concentration was estimated as 191.76mg/l (Fig. 3).

Table 1
Behavioural and dermatological changes of *Clarias gariepinus* juveniles exposed to varied concentrations of *Carica papaya* seed extract during acute and sub-lethal phases

ACUTE TEST																
Exposure time (hour)	**24**				**48**				**72**				**96**			
Concentration (mg/L)	**0**	**150**	**225**	**300**	**0**	**150**	**225**	**300**	**0**	**150**	**225**	**300**	**0**	**150**	**225**	**300**
Behavioural changes																
Loss of reflex	-	+	+	+	-	++	++	++	-	++	++	++	-	+++	+++	+++
Air gulping	-	-	+	+	-	++	++	++	-	++	++	++	-	+++	+++	+++
Erratic swimming	-	-	-	+	-	-	+	+	-	+	+	++	-	++	++	+++
Dermatological changes																
Discoloration	-	+	+	+	-	++	++	++	-	++	++	++	-	+++	+++	+++
Haemorrhage	-	-	-		-	-	-	-	-	+	+	+	-	++	++	++
Sub-lethal test																
Exposure time (hour)	**48**				**96**				**144**				**168**			
Behavioural changes																
Loss of reflex	-	-	-	-	-	-	-		-	-	-	+	-	-	-	+
Air gulping	-	-	+	+	-	-	+	+	-	+	+	+	-	+	++	+++
Erratic swimming	-	-	-	-	-	-	-	-	-	-	-	-	-	-	++	++
Dermatological changes																
Discoloration	-	+	+	+	-	+	+	+	-	+	+	++	-	+	++	+++
Haemorrhage	-	-	-		-	-	-	-	-	-	-		-	-	-	-

Where - = no significant sign, + = low severity, ++ = moderate severity and +++ = high severity.

Table 2
Percentage mortality of *Clarias gariepinus* juveniles exposed to varied concentrations of *Carica papaya* seed extract during acute phases

TOXICANT CONCENTRATION (MG/L)	**PERCENTAGE MORTALITY**				
	24h	**48h**	**72h**	**96h**	**Cumulative mortality (%)**
0	0.00	0.00	0.00	0.00	0.00
150	6.67	13.33	20.00	6.67	46.67
225	13.33	13.33	26.67	13.33	66.67
300	20.00	20.00	33.33	20.00	93.33
LC_{50} (mg/l)	1200.78	426.67	191.76	163.02	-
Log (concentration)	3.08	2.63	2.28	2.21	-

RECENT SCIENCE Publishing Research
© RECENT SCIENCE PUBLICATIONS ARCHIVES| August 2013|$25.00 | 27702685 |
This article is authorized for use only by Recent Science Journal Authors, Subscribers and Partnering Institutions

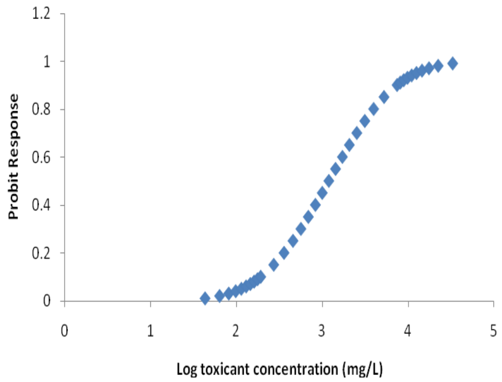

Fig. 1 Probit transformed responses for 24h exposure of *Clarias gariepinus* exposed to graded concentrations of *Carica papaya* seed aqueous extract

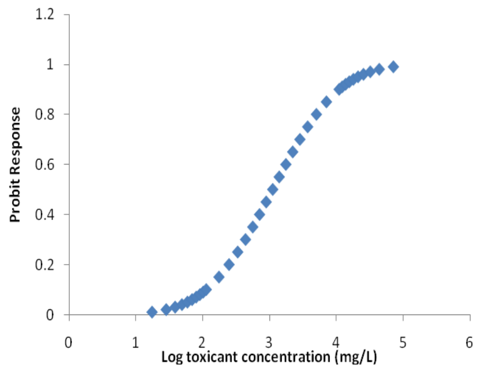

Fig. 2 Probit transformed responses for 48h exposure of *Clarias gariepinus* juveniles exposed to graded concentrations of *Carica papaya* seed aqueous extract

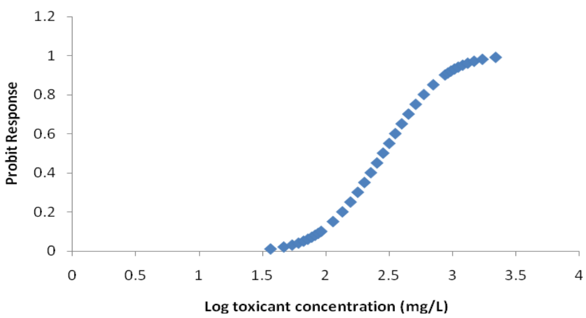

Fig. 3 Probit transformed responses for 72h exposure of *Clarias gariepinus* exposed to graded concentrations of *Carica papaya* seed aqueous extract

The percentage mortality at 96h increased with the toxicant concentration. Catfish juveniles exposed to 150, 225 and 300mg/l had 6.67, 13.33 and 20.00% mortalities, respectively (Table 2). The LC_{50} at 95% confidence limit was estimated as 163.02mg/l (Fig. 4). The models explain the data were highly efficient at 97.6%.

3.3 Liver Enzymes

The ACP levels in the liver tissues were significant higher ($P \leq 0.05$) at 72 and 144h when compared to 0h.

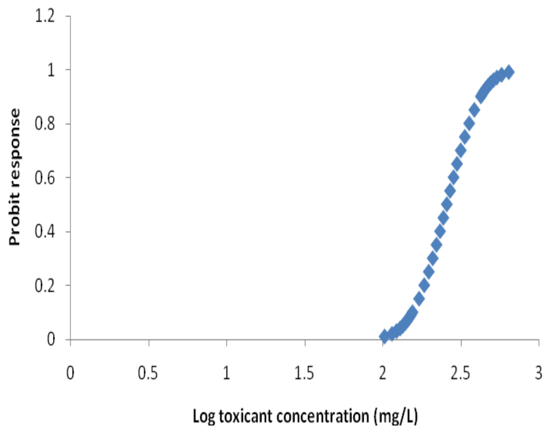

Fig. 4 Probit transformed responses for 96h exposure of *Clarias gariepinus* juveniles exposed to graded concentrations of *Carica papaya* seed aqueous extract

The AST activity in the liver of *C. gariepinus* juveniles exposed to *C. papaya* seed extract increased from 0 to 72 hours was highest at 144h in 75 and 100mg/l *C. papaya* seed extract treatments. For exposure period of 72h, AST activity in the liver was highest for 50, 100 and 75mg/l C. papaya seed extract treatments. The ALT enzyme activity in the liver of *C. gariepinus* exposed to *C. papaya* seed extract was dose and time dependent. The liver ALT activity in all the treatments increased from 0 to 72 and 144h. Furthermore, the ALT activity increased progressively with increase in toxicant concentration. The mean values were statistically significant ($p \leq 0.05$) (Table 3).

The ACP enzyme activity in the liver of *Clarias gariepinus* decreased gradually from with exposure time except for the control. The mean values of ACP enzyme activity for the duration of exposure (hours) were statistically significant ($p<0.05$). Furthermore, the AST enzyme activity increased sharply across the exposure periods. Also, the mean value of AST enzyme activity at 0h was statistically significant to 72h and 144h ($p \leq 0.05$), whereas, the mean value of AST enzyme activity at 72h and 144h were not statistically significant ($p>0.05$). However, the ALT enzyme activity in the liver increased progressively with exposure time. Also, the mean values of ALT enzyme activity is statistically significant to 72h and 144h ($p<0.06$), whereas, the mean value at 72h and 144h of ALT enzyme activity are not statistically significant ($p \geq 0.05$).

4. DISCUSSION

Bioassay of toxicant occupies a central place in aquatic ecotoxicology. The aim of such test is to determine the critical amount of toxicants or their mixtures that can be tolerated by the aquatic organisms and to predict the influence of the toxicant. The present study was not the first toxicological test of pawpaw powder extracts to aquatic animals. Data obtained from this study showed that percentage mortality of *C.gariepinus* juveniles

RECENT SCIENCE

© RECENT SCIENCE PUBLICATIONS ARCHIVES| August 2013|$25.00 | 27702685 |
This article is authorized for use only by Recent Science Journal Authors, Subscribers and Partnering Institutions

Table 3
Mean value of enzymes activity in liver of *Clarias gariepinus* exposed to *Carica papaya* seed aqueous extract

DOSAGE (MG/L)	EXPOSURE DURATION (HOURS)		
	0	72	144
	Acid phosphatase **(ACP)**		
0	$2.142351 \pm 0.27^{a*}$	$1.236254 \pm 0.508^{a*}$	$1.44767 \pm .034^{a*}$
50	$3.40200 \pm 0.34^{b**}$	$3.13000 \pm 0.577^{c**}$	$1.24933 \pm .092^{a*}$
75	$4.51633 \pm 0.86^{c**}$	$1.07667 \pm 0.033^{a*}$	$1.28300 \pm .044^{ab*}$
100	$3.49067 \pm 0.87^{b***}$	$2.01967 \pm 0.058^{b**}$	$1.48467 \pm .039^{ab*}$
	Aspartate transferase (AST)		
0	$12.91 \pm 1.00^{c*}$	$12.95 \pm 1.00^{a*}$	$13.03 \pm 1.73^{a*}$
50	$10.00 \pm 1.01^{b*}$	$23.00 \pm 0.00^{c***}$	$17.00 \pm 1.00^{b**}$
75	$10.53 \pm 0.57^{b*}$	$19.00 \pm 0.00^{b**}$	$18.00 \pm 1.00^{b**}$
100	$9.70 \pm 0.39^{a*}$	$23.00 \pm 0.00^{c**}$	$36.00 \pm 0.00^{c***}$
	Alanine transferase (ALT)		
0	$5.72 \pm 0.00^{a*}$	$5.83 \pm 1.33^{a*}$	$5.72 \pm 0.00^{a*}$
50	$7.00 \pm 0.31^{c**}$	$5.33 \pm 1.33^{a*}$	$8.00 \pm 2.31^{b***}$
75	$6.33 \pm 0.38^{b*}$	$6.67 \pm 1.33^{b*}$	$10.67 \pm 1.33^{c**}$
100	$6.40 \pm 0.60^{b*}$	$10.67 \pm 1.33^{c**}$	$11.00 \pm 1.00^{c**}$

Mean values having the same alphabets as superscripts along the column do not show significant difference ($p \geq 0.05$). Mean values having the same asterisk as superscripts along the row do not show significant difference ($p \geq 0.05$).

increased with increase in concentration of *C. papaya* and was dose dependent. The observed values for catfish juveniles mortality was in agreement with those of Ayotunde and Offem [5,6] for Nile tilapia. Acute toxicity occurred at concentrations comparable to those of lead [10], diazinon [11], phenol [12] and tetrachloromethane [13] but lower than those of benzene [14], methanol [15] and acetonitrile [16]. However, pawpaw powder was less toxic than chlorine [17] and ammonia [18]. For such comparison to be meaningful, species variability and possible differences in water quality needs to be accounted for. The latter is important, since hardness, alkalinity and pH of the medium can all influence the species and the extent of toxicity [19,20]. However, because the same medium was used, changes in the effective toxicant concentration due to possible interaction with the medium were ruled out. In this experiment, the 96 h LC_{50} value of aqueous extracts of pawpaw seed powder to *C. gariepinus* juveniles was higher than the value obtained Nile tilapia fingerlings exposed to similar concentrations of pawpaw seed aqueous extract [5,6]. The difference in toxicity may be species and size specific. In a similar experiment with organochlorine substances, Albaiges et al. [21] revealed that the levels of chemicals in the gonads and liver of fish were similar in both adults and young fishes, which indicated that the age and thus size of fish was a significant factor in the accumulation of toxicants. However, these results disagreed with the size-specific sensitivity to acute chemical toxicity observed in some aquatic animals with the smallest individuals showing the highest sensitivity [22,23]. The size-specific and interspecific difference in lethal level will allow the effective usage of pawpaw seed as anti-fertility agent in tilapia polyculture with catfish [5,6]. *Clarias sp* is ecologically adapted to muddy environments in which temporary changes in water chemistry are more rapid and the contaminant concentration are usually higher. Such an environmental stress may facilitate tolerance to increased concentrations of contaminants [24]. This view was supported by the observation, which revealed that 96 h may not to be sufficient time to determine the asymptotic LC_{50} for the pawpaw seed powder concentration to Clarias fingerlings since mortality would have continued if exposure time was extended. Three factors for the selective toxicity of toxicants for various fish species such as: different inhibition of acetyl cholinesterase, different detoxification and absorption has been suggested [25]. The above factors may probably be responsible for the different reactions showed by catfish fingerlings in response to varying concentration of aqueous extracts of *C. papaya* seed. The reactions were more pronounced at higher concentration due to increased inhibition of acetylcholinesterase which eventually results in the death of the fish [11,26-29]. In toxicological experiments, the time of exposure has large effect on biological response. The general rule of thumb is that the longer the exposure time, the lesser the LC_{50} value and the greater the toxicity. Results of this study showed similar pattern having lesser 96h LC_{50} than 48h LC_{50} and so on, with increasing ratios of 24:48h, 24:72h and 24:96h LC_{50} as 2.81, 6.26 and 7.37 indicating delayed acute toxicity response. Dose-response approach in estimating the lethal effects of toxicants on organisms have been criticized for lacking real ecological meaning [30,31]. Nonetheless, regulatory norms have been built around LC_{50} values that can be compared across toxicants and organisms [13]. Thus, LC_{50} values from dose-response bioassays have become the starting points for ecologically relevant studies of toxicant effects on animal populations [32].

Liver ACP levels were significantly decreased in *C. papaya* treated fish at various concentrations. The ACP is an inducible enzyme because its activity goes up when there is a toxic impact and the enzyme begins to

RECENT SCIENCE Publishing Research

© RECENT SCIENCE PUBLICATIONS ARCHIVES| August 2013|$25.00 | 27702685 |
This article is authorized for use only by Recent Science Journal Authors, Subscribers and Partnering Institutions

counteract the toxic effect [33]. Subsequently, the enzyme may begin to drop either as a result of having partly or fully encountered the toxin or as a result of cell damage. Alteration in the membrane permeability can have severe consequences such as leakage of hydrolytic enzyme including ACP, which would have detrimental effect on the cell. However, if due to toxicity of a substance, there is increased ACP activity, then it means that the substance interacted with the lysosome and caused an increase in the lysosomal activity in the liver [33].

Liver AST and ALT were significantly increased in *C. papaya* treated fish, though not all exposure days were statistically significant. This indicated that *C. papaya* stimulates glutamate transaminase activity in the liver which could be due to injury caused by *C. papaya*, which may stimulate tissue repair through protein turn over and increased respiration.

5. CONCLUSIONS

The pawpaw seed powder had a positive toxicity effect correlating with exposure time from 24 to 96h on *C. gariepinus*. From the toxicity tests pawpaw seed powder concentration as low as 163.02mg/l can be potentially hazardous to some freshwater fish. Therefore, acute toxicity data of the present study provide baseline information needed to develop models on the use of pawpaw seed powder as piscidal agent.

ACKNOWLEDGEMENT

We are indebted to the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka for providing laboratory space and facilities for the study. We are also thankful to Mr. and Mrs. Levi Echefu who funded the study. There is no conflict of interest among the authors.

REFERENCES

[1] P. O. Akah, A. N. Oli, N. M. Enwerem, and K. Gamaniel, “Preliminary studies on purgative effect of *Carica papaya* root extract,” Fitoterapia, vol. 68(4), pp. 327–331, Jan. 1997.

[2] A. E. Eno, O. I. Owo, E. H. Itam, and R. S. Konya, “Blood pressure depression by the fruit juice of *Carica papaya* (L.) in renal and DOCA-induced hypertension in the rat,” Phytotherapy Research, vol. 14(4), pp. 235–239, Jun. 2000.

[3] R.K.Wilson, T. K. Kwan, C. Y. Kwan, and Sorger, G. J. “Effects of papaya seed extract and benzyl isothiocyanate on vascular contraction,” Life Sciences, vol. 71, pp. 497–507, 2002.

[4] N. K. Lohiya, P. K. Mishra, N. Pathak, B. Manivannan, and S. C. Jain, “Reversible azoospermia by oral administration of the benzene chromatographic fraction of the chloroform extract of the seeds of *Carica papaya* in rabbits,” Advances in Contraception, vol. 15(2), pp. 141–161, Jun. 1999.

[5] E.O.Ayotunde, and B.O.Ofem, “Acute and chronic toxicity of pawpaw (*Carica papaya*) seed powder to Nile tilapia (Oreochromis niloticus Linne 1757) fingerlings,” Journal of Agricultural Technology and Environment, vol. 1, pp. 1–4, 2005.

[6] E.O.Ayotunde, and B.O.Ofem, “Acute and chronic toxicity of pawpaw (*Carica papaya*) seed powder to adult Nile tilapia (Oreochromis niloticus Linne 1757). African Journal of Biotechnology, vol. 7(13), pp. 2265–2274, Jul. 2008.

[7] A.L.Babson, and P.A. Read, “A new assay for prostatic acid phosphatase in serum,” American Journal of Clinical Pathology, vol. 32(1), pp. 89–91, Jul. 1959.

[8] S.Reitman, and S.Frankel, “A colorimetric method for the determination of oxaloacetic acid glutamic pyruvic transaminase,” American Journal of Chemical Pathology, vol. 28, 53–56, 1957.

[9] D.J.Finney, Probit Analysis, 3rd ed., London, England: Cambridge University Press, 1971.

[10] A. A. Oladimeji, and B. O. Offem, “Toxicity of lead to Clarias lazera, Oreochromis niloticus, Chironomus tentans and Benacus sp.,” Air, Water, and Soil Pollution, vol. 44(3-4), pp. 191–201, Apr. 1989.

[11] O. B. Adedeji, A. O. Adedeji, O. K. Adeyemo, and S. A. Agbede, “Acute toxicity of diazinon to the African catfish (*Clarias gariepinus*),” African Journal of Biotechnology, vol. 7(5), pp. 651–654, Mar. 2008.

[12] U. M. Cowgill, and D. P. Milazzo, “The sensitivity of *Ceriodaphnia dubia* and *Daphnia magna* to seven chemicals utilizing the three-brood test,” Archives Environmental Contamination and Toxicology, vol. 20, pp. 211–213, Feb. 1991.

[13] G.A.LeBlanc, “Acute toxicity of priority pollutants to water fleas (*Daphnia magna*),” Bulletin of Environmental Contamination and Toxicology, vol. 24(5), pp. 684–691, May 1980.

[14] J.H.Canton, and D.M. M.Adema, “Reproducibility of short-term and reproduction toxicity experiments with *Daphnia magna* and comparison of the sensitivity of *Daphnia magna* with *Daphnia pulex* and *Daphnia cucullata* in short-term experiments,” Hydrobiologia, vol. 59(2), pp. 135–140, Jun. 1978.

[15] Z. Tong, Z. Huailan, and J. Hongjun, “Chronic toxicity of acrylonitrile and acetonitrile to *daphnia magna* in 14-d and 21-d toxicity tests,” Bulletin of Environmental Contamination and Toxicology, vol. 57, pp. 655–659, 1996.

[16] L.Guilhermino, T. Diamantino, M. C. Silva, and A. M. Soares, “Acute toxicity test with *Daphnia magna*: an alternative to mammals in the

© RECENT SCIENCE PUBLICATIONS ARCHIVES| August 2013|$25.00 | 27702685 |
This article is authorized for use only by Recent Science Journal Authors, Subscribers and Partnering Institutions

prescreening of chemical toxicity?," Ecotoxicology and Environmental Safety, vol. 46(3), pp. 357–362, Jul. 2000.

[17] M.Kaniewska-Prus, "The effect of ammonia, chlorine, chloramines toxicity on the mortality of *Daphnia magna* Straus," Polish Archives of Hydrobiology, vol. 29(3-4), pp. 607–624, 1982.

[18] D. I. Mount, and T. J. Norberg, "A seven-day life cycle cladoceran toxicity test," Environmental Toxicology and Chemistry, vol. 3(3), pp. 425–434, Jul. 1984.

[19] C. Barata, D. J. Baird, and S. J. Markich, "Influence of genetic and environmental factors on the tolerance of *Daphnia magna* Straus to essential and nonessential metals," Aquatic Toxicology, 42(2), 115–136, Jun. 1998

[20] D.G.Heijerick, C. R. Janssen, and W. M. De Coen, "The combined effects of hardness, pH, and dissolved organic carbon on the chronic toxicity of Zn to D. *magna*: development of a surface response model," Archives Environmental Contamination and Toxicology, vol. 44(2), pp. 210–217, Feb. 2003.

[21] J. Albaiges, A Farran, M. Soler, A. Gallifa, and P. Martin,"Accumulation and distribution of biogenic and pollutant hydrocarbon, PCB and DDT in tissues of Western Mediterranean fishes," Marine Environmental Research, vol. 22(1), pp. 1– 18, 1987.

[22] R. Goyer, and T. Clarkson, Toxic Effects of Heavy Metals, C. Klaassen, Ed. Casarett Doull's Toxicology, New York: McGraw- Hill, 2001.

[23] A.Bianchini, M. Grosell, S. M. Gregory, C. M. Wood, "Acute silver toxicity in aquatic animals is a function of sodium uptake rate," Environmental Science and Technology, vol. 36(8), pp. 1763–1776, Apr. 2002.

[24] S. Koivisto, "Is Daphnia magna an ecological representative zooplankton species toxicity test," Environmental Pollution, vol. 90(2), pp. 263–289, 1995.

[25] H. S. Oh, S. K. Lee, Y. H. Kim, and J. K. Roh, "Mechanism of selective toxicity of diazinon to killifish (*Oryzias latipes*) and loach (*Misgurnus anguillicaudatus*)," M. A. Mayes and M. G. Barron, (Eds.), Aquatic Toxicology and Risk Assessment, vol. 14, pp. 343–353, 1991.

[26] M.Muniyan, and K. Veeraragghavan, "Acute toxicity of ethofenprox to the freshwater fish Oreochromis mossambicus (Peters)," Journal of Environmental Biology, vol. 20(2), pp. 153–155, 1999.

[27] M.Santhakumar, and M. Balaji, "Acute toxicity of an organophosphorus insecticide monocrotophos and its effects on behaviour of an air-breathing fish, Anabas testudineus Bloch," Journal of Environmental Biology, vol. 21(2), pp. 121–123, 2000.

[28] V. O. Ayuba, and P. C. Ofojekwu, "Acute toxicity of the Jimson's weed (*Datura innoxia*) to the African catfish (*Clarias gariepinus*) fingerlings," Journal of Aquatic Science, vol. 17: pp. 2–12, 2002.

[29] C. M. Liao, B. C. Chen , S. Singh, M. C. Lin, C. W. Liu, and B. C. Han, "Acute toxicity and bioaccumulation of arsenic in tilapia (*Oreochromis mossambicus*) from a blackfoot disease area in Taiwan," Environmental Toxicology, vol. 18(4), pp. 252–259, Aug. 2003.

[30] C. Newman, Quantitative Methods in Aquatic Toxicology. Florida, USA: Lewis Publishers, 1995.

[31] J.Pernak, K.Sobaszkiewicz, and I. Mirska, "Anti-microbial activities of ionic liquids," Green Chemistry, vol. 5, pp. 52–56, Jan. 2003.

[32] D. W. Schindler, "Detecting ecosystem responses to anthropogenic stress," Canadian Journal of Fisheries Aquatic Science, vol. 44(S1), pp. s6–s25, Dec. 1987.

[33] N. Ghorpade, V. Mehta, M. Khare, P. Sinkar, S. Krishnan, and C. V. Rao, "Toxicity study of diethyl phthalate on freshwater fish *Cirrhina mrigala*," Ecotoxicology and Environmental Safety, vol. 53(2), pp. 255–258, Oct. 2002.

RECENT SCIENCE Publishing Research

© RECENT SCIENCE PUBLICATIONS ARCHIVES| August 2013|$25.00 | 27702685 |
This article is authorized for use only by Recent Science Journal Authors, Subscribers and Partnering Institutions