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Biochemical Parameters of Common Carp (*Cyprinus carpio*) Exposed to Crude Leaf Extract of *Cannabis sativa*

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Abstract

The effect of sub-lethal concentrations (1.88, 3.75, 7.50, 15.00 and 30.00 mg/L) of crude leaf extract of *Cannabis sativa* was determined in the plasma, liver and gill biochemical parameters of Common carp, *Cyprinus carpio* (mean weight of 15.05±0.05g) after 56-day exposure period in static renewable bioassay system. During the experiment, some physico-chemical parameters were monitored while at the end of the experiment, the selected biochemical parameters were determined in the plasma, liver and gill of the test fish. The biochemical parameters determined were alkaline phosphatase (ALP), Lactate dehydrogenase (LDH), total protein (TP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin, total bilirubin (TB), albumin (ALB), urea acid (UA) and cholesterol (CHOL). The monitored pH showed significant difference (p<0.05) while water temperature, total alkalinity, dissolved oxygen and free carbon (iv) oxides showed no significant difference (p>0.05) in *C. sativa* test set-ups compared to the control during the experimental period. There were significant difference (p<0.05) in some of the determined biochemical parameters of the test fish exposed to *C. sativa* compared to the control. Therefore, it can be deduce from the study that prolonged exposure of *C. carpio* fingerlings to crude leaf extract of *C. sativa* affected some determined biochemical parameters in the tissue/organs of the test fish.

Keywords: *: Cannabis Sativa, Biochemical Parameters, Cyprinus Carpio .*

1. Introduction

Cannabis sativa is a cosmopolitan weedy plant that is grown in many parts of the world. It contains compounds with active ingredients of varying potencies such as tetrahydrocannabinol (THC) (Hampson *et al*., 2000; Koch, 2001), phytocannabinoids and plant steroids (Rifat, *et al.,* 2010; Audu *et al*., 2013). The phytochemical analysis of the leaves revealed the presence of alkaloid, flavonoids, cardiac glycosides, resins, terpenes and steroids (Audu *et al.,* 2013). Studies conducted by Amna (2011) revealed that *C. sativa* caused significance difference in certain clinical enzymes in rats and men. Studies have also noted the potency of cannabis as pest repellent and pesticides of potato beetle - *Leptinotarsa decemlineata* (Stratii, 1976), wheat root maggot-*Delia coarctata* (Pakhomov and Potushanskii, 1977) and root exudates of European chafer-*Melolontha melolontha* (Mateeva, 1995). The plant has been reported to have anesthesia effect on *Oreochromis niloticus* (Audu *et al.,* 2013).

Due to the fact that anaesthetics are used with increasing frequency in aquaculture, mainly to reduce the stress and to prevent mechanical damage to fish during handling as their use is particularly common in stripping, marking, health checks, etc (Ross and Ross 1999), there is the need to study their effect on the biochemical parameters of fish. The common carp (*Cyprinus carpio*) belongs to the family cyprinidae and is one of the most important breeder species (Mohamad *et al*., 2011) with an increasing farmers' preference. The fish is an economically significant species. It is cultivated commercially (Cao *et al.,* 2013) in other parts of the world including Austrialia and South America and Africa because of its fast growth rate, facile cultivation and high feed efficacy ratio (Tokur *et al.,* 2006). According to Jalali Mottahari *et al*. (2013) it is a potential species for fish culture since it can tolerate a wide range of changing water pH. The fish has gained researchers interest as test fish in conducting toxicity test with natural products (Selamoglu *et al*., 2012).

C. sativa have various applications with their attendant side effects, however, there is no scientific documentation of the effects of the crude leaf extract on the plasma, liver and gill biochemical parameters of *C. carpio*. Therefore, this study was undertaken to determine the effects of the crude leaf extract of *C. sativa* on the plasma, liver and gill biochemical parameters of *C. carpio* after the 56-day experiment in static renewable bioassay system.

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2. Materials and Methods

Marijuana (*Cannabis sativa* (L.)) was obtained from the National Drug Law Enforcement Agency (NDLEA), Jos command, Plateau State, Nigeria, strictly for scientific research. The leaves were carefully sorted out from the stem/twigs by handpicking, ground into powder then sieved through a metal sieve (90µm mesh size) and stored in airtight polyethylene bag for use.

A stock solution of the crude leaf extracts was prepared by macerating 12.5g of the dried powdered leaves in 500ml of petroleum ether for 24 hours at 25^{o}C. From this oily (950mg petroleum ether free) resin, the concentrations used for the experiment were prepared. These concentrations were obtained from the value of 96 h LC50 as 30(I/3rd of LC50), 15 (1/6th of LC50), 7.50(1/12th of LC50), 3.75(1/24th of LC50) and 1.88mg/L (1/48th of LC50). These were placed into clean and dry conical flasks where 10, 20, 40, 80 and 100 ml of acetone was added respectively to give the appropriate concentrations used for the test. Water and acetone in water were used as control as described in UNEP (1989). A total of ten fish were distributed in each test concentration and controls in aquaria (60x40x40cm) with two replications.

Two hundred fingerlings of *C. carpio* average weight of 15.05 ±0.05g were obtained from the extension unit of Bauchi State Agricultural Development Project (BSADP) Bauchi State, Nigeria and transported to the Applied Hydrobiology and Fisheries Laboratory of University of Jos, Nigeria, in an oxygenated polyethylene bags. They were held in rectangular tanks and allowed to acclimatize for two weeks. During the acclimatization and exposure periods, the fish were fed to satiation with 3mm commercially pelleted fish feed (Multifeeds®; Protein 42%, Fat12%, Ash7.5% Fiber2.6%). Dechlorinated tap water was used during acclimatization and exposure periods. The experimental aquaria were supplied with continuous dissolved oxygen through a giant aeration pump. Physico- chemical parameters were monitored throughout the 56- day trial by the methods described in APHA *et al*. (1985). At the end of 56 day exposure period, the plasma, gills, and liver of exposed and controls fish were collected and examined for the level of alkaline phosphatase (ALP), lactate dehydrogenase (LDH) total protein (TP) albumin (ALB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin (BIL), total bilirubin (TBIL), uric acid (UA) and cholesterol (CHOL). Blood collection was through cardiac puncture using (1ml) non heparinised syringe and needle. The blood was immediately centrifuged at 1500 rpm for 5 minutes to obtain the plasma. The gills and liver samples were homogenized in buffer (0.25M sucrose, 0.01M TRIS and 0.01M EDTA) and centrifuged at 1500 rpm for 10 minutes where the supernatant was used for the analyses. The activities of ALP, LDH, TP, AST, ALT, BIL, TBIL, UA and CHOL were assayed with the aid of GENESYS-20 spectrophotometer (GENESYS 20.Thermo Electron Corporation USA) following the manufacturer's instruction of the of Randox, Spectrum and Fortress reagent kits.

Data obtained were analyzed for means, standard error, analysis of variance (ANOVA) and Duncan's multiple range tests for significance difference at 5% level of probability using the statistical package SPSS 17.0 computer program (SPSS Inc. Chicago, Illinois, USA).

3. Results

The monitored physico-chemical parameters of the experimental set-up are presented in Table 1. There were no significant difference (p>0.05) in all the monitored physico-chemical parameters except pH. As the concentrations of the C. sativa increased the test medium becomes acidic with the test medium (30.00 mg/L) recording the pH of 6.20 (0.45). There was no significant difference (p>0.05) recorded in the monitored physico-chemical parameters between the two control set-ups. The activities of ALP and LDH in the sampled tissue/organs of the test fish were presented in Table 2. As the concentrations of the plant leaf extract increased the activity of ALP and LDH in the plasma, gill and liver significantly decreased and increased (p<0.05) respectively after the 56-day experimental period. LDH and ALP activities in the control groups were observed to be highest in the gill, less in the liver and least in the plasma.

The activities of AST, ALT, ALB, TBIL, BIL, UA and CHOL in the plasma of the test fish exposed to the plant leaf extract were presented in Table 3. As the concentrations of the plant extract increased the activity of AST, ALT and UA increased while those of ALB, TBIL, BIL and CHOL were decreased. There was significant difference (p<0.05) in the plasma activities of ALT, UA and CHOL after the 56-day experimental period. The determined values of total proteins in plasma and liver of the test fish exposed to sublethal concentrations of the plant leaf extract were presented in Table 4. There were no significant difference (p>0.05) in the plasma and liver total protein level after the 56-day experimental period.

Table 1. Physico-chemical parameters of experimental set-up used to study the effect of *C. sativa* on the biochemical parameters of *C. carpio* during the 56-day experimental period

Physico-chemical parameters	**Concentration of *C. sativa* (mg/L)**						
	0.00 (Water)	**0.00 (Acetone)**	**1.88**	**3.75**	**7.50**	**15.00**	**30.00**
Temperature (°C)	22.40 (0.55)	22.40 (0.55)	22.40 (0.55)	22.40 (0.55)	22.40 (0.55)	22.40 (0.55)	22.40 (0.55)
pH	7.00 (0.00)	7.00 (0.00)	6.60 (0.31)	6.60 (0.22)	6.40 (0.41)	6.20 (0.45)*	6.20 (0.45)*
Dissolved oxygen (mg/l)	4.50 (0.00)	4.50 (0.00)	4.50 (0.00)	4.10 (0.22)	4.10 (0.22)	4.10 (0.22)	4.00 (0.22)
Total Alkalinity (mg/l)	30.25 (0.49)	30.31 (0.27)	32.38 (1.54)	32.87 (1.68)	34.02 (2.03)	35.48 (3.15)	36.60 (3.67)
Free carbon (iv) oxides (mg/l)	4.33 (0.16)	4.31 (0.18)	4.41 (0.22)	4.49 (0.31)	4.68 (0.22)	4.93 (0.43)	5.18 (0.48)

Standard deviation in parenthesis * $p<0.05$ across the row compared to the control

Table 2. Alkaline Phosphatase and Lactate Dehydrogenase activities in *Cyprinus carpio* Exposed to Sublethal Concentrations of *C. sativa* Crude leaf extract after 56 days

Parameters	**Tissue/Organs**	**Concentrations of *C. sativa* (mg/L)**						
		0.00 (Water)	**0.00 (Acetone)**	**1.88**	**3.75**	**7.50**	**15.00**	**30.00**
Alkaline Phosphatase (U/L)	Plasma	105.66 (3.45)	117.62 (2.51)	86.71 (3.91)	63.99 (5.29)*	59.99 (3.81)*	59.27 (4.29)*	51.631 (7.26)*
	Liver	175.07 (3.71)	163.62 (7.41)	152.99 (8.12)	128.53 (2.54)	120.72 (9.43)	85.81 (7.34)*	76.36 (4.53)*
	Gill	211.61 (1.69)	197.07 (8.56)	185.98 (8.05)	146.53 (1.67)	127.26 (2.28)*	125.26 (1.63)*	121.26 (3.44)*
Lactate Dehydrogenase (U/L)	Plasma	592.92 (5.21)	642.42 (6.43)	1210.05 (102.45)*	1250.39 (98.56)*	1730.03 (124.92)*	1922.09 (186.56)	2134.45 (321.76)*
	Liver	610.35 (34.61)	613.90 (75.09)	648.76 (30.18)	728.48 (112.09)	748.82 (96.28)	809.82 (106.48)*	884.38 (34.76)*
	Gill	609.06 (23.57)	669.08 (46.28)	665.55 (29.71)	649.41 (31.33)	866.61 (63.02)*	878.51 (39.48)*	933.12 (83.42)*

* $p<0.05$ across the row compared to the controls, standard deviation in parenthesis

Table 3. Activities of Serum Biochemical of *Cyprinus carpio* Exposed to Sublethal Concentrations of *C. sativa* Crude leaf extract after 56 days

Parameters	Concentrations of *C. sativa* (mg/L)						
	0.00 (Water)	0.00 (Acetone)	1.88	3.75	7.50	15.00	30.00
Aspartate aminotransferase (U/L)	293.67 (12.58)	294.00 (12.43)	295.17 (17.28)	320.83 (23.11)	322.67 (23.62)	326.80 (30.19)	385.33 (38.28)
Alanine aminotransferase (U/L)	13.35 (1.05)	16.67 (1.56)	25.67 (2.37)	28.00 (4.28)*	30.33 (4.61)*	34.00 (5.39)*	35.65 (6.82)*
Albumin (g/dl)	1.57 (0.01)	1.33 (0.02)	1.14 (0.01)	1.20 (0.01)	1.07 (0.02)	1.00 (0.03)	1.00 (0.06)
Bilirubin (mg/dl)	1.04 (0.00)	1.05 (0.00)	0.98 (0.00)	0.91 (0.00)	1.05 (0.01)	1.07 (0.01)	1.05 (0.01)
Total Bilirubin (mg/dl)	0.78 (0.00)	0.73 (0.00)	0.72 (0.00)	0.85 (0.00)	0.80 (0.00)	0.79 (0.01)	0.76 (0.01)
Uric acid (mg/dl)	71.11 (1.27)	76.40 (1.12)	71.08 (0.45)	88.33 (1.45)	114.92 (4.32)	130.54 (3.62)*	136.39 (4.72)*
Cholesterol (mg/dl)	432.87 (12.65)	385.47 (14.49)	113.95 (17.49)*	114.17 (21.69)*	213.80 (12.62)*	141.89 (12.56)*	112.52 (10.49)*

* $p<0.05$ across the row compared to the controls, standard deviation in parenthesis

Table 4. Total protein values of *Cyprinus carpio* Exposed to Sublethal Concentrations of *C. sativa* Crude leaf extract after 56 days

Parameters	Tissue/Organs	Concentrations of *C. sativa* (mg/L)						
		0.00 (Water)	0.00 (Acetone)	1.88	3.75	7.50	15.00	30.00
Total protein (g/dl)	Plasma	4.63 (1.23)	5.96 (0.89)	3.55 (2.48)	4.63 (2.07)	5.39 (0.94)	5.58 (1.08)	4.57 (1.09)
	Liver	3.30 (0.45)	3.30 (0.36)	3.68 (0.41)	3.87 (0.89)	5.58 (0.02)	5.58 (0.01)	5.58 (0.46)

Standard deviation in parenthesis

4. Discussion

The significant decrease in static renewable bioassay system pH as the concentrations of *C. sativa* increased, according to Adamu (2009), is attributed to the production of acidic metabolites by the plant leaf extract which Aleem (1987) suggested that the acidic condition of the water resulted to the decrease in the level of dissolved oxygen. However, Jalali Mottahari *et al*. (2013) reported that *C. carpio* are very tolerant to wide range of pH value thus the pH level recorded in this study may not be a threat to the test fish. The insignificant decrease in the level of dissolved oxygen may be attributed to the use of aeration during the study period. The temperature range recorded in this study were within the range for the fish as Mahdavi *et al*. (2013) reported 20 - 28°C for the culture of the test fish. Therefore, the temperature was within acceptable limits for fish culture (Swann, 2006). According to Capkin *et al*. (2006), total alkalinity above 20mg/L can significantly increase the survival rate of fishes thus the higher total alkalinity may be responsible for the 100% survival rate recorded in this study.

The value of total protein (3.55 (2.48) - 5.96 (0.89)) and albumin (1.00 (0.06) - 1.57 (0.01)) reported in the plasma of the test fish were within the range reported by Selamoglu *et al*. (2012) showing that the physiology of the normal fish is not affected by the exposure of the test fish to *C. sativa*. According to Adamu *et al*. (2013), the insignificant increase in liver total protein as the concentration of the plant extract increased may be due to a high demand of protein to metabolize the plant content or possibly due to haemo-concentration arising from fluid loss (Awasthy *et al.,* 2010). The increase liver total protein may also be attributed to the need to utilize protein as an energy source to compensate for increased energy demand to cope with leaf extract-induced stress (Dogan and Can, 2011). However, the decrease in plasma albumin recorded in this study may impede its function of transportation (Adamu and Kori-Siakpere, 2011) which may have resulted from the inhibitory effect of the plant on protein hydrolytic activity due to protease activity which corresponds to the decrease liver total protein level.

The hypocholesteremia condition recorded in the test fish exposed to plant leaf extract in this study is reported by Adamu and Kori-Siakpere (2011) in hybrid catfish exposed to tobacco leaf dust. Cholesterol level decreased as the concentration of the plant leaf extract increased which is accord with the findings of Samson *et al.* (2011). Alaa *et al*. (2010) asserted that cholesterol is the most important sterol occurring in plasma and red blood cells. If this assertion be true then, it is logical to add that the decrease in RBC content due to increased sublethal concentrations of *C. sativa* resulted in decrease cholesterol level in the blood of the exposed fish.

According to Martin *et al.* (1983), aminotransferase links carbohydrate and protein metabolism as it catalyzes their inter-conversion. The activities of these enzymes are directly proportional to the level of total protein and inversely proportional of cholesterol, an indication that the enzymes are catalyzing the inter-conversion of carbohydrate to protein in the liver. The activities of plasma aminotransferases were within the range reported by Selamoglu *et al*. (2012) in the test fish. It therefore noted that *C. sativa* caused increase in the activities of aminotransferases, as Amna (2011) reported increase in ALT and AST activities in rats exposed to *C. sativa*, which was concentration dependent. This therefore revealed that the plant has the potential of causing liver dysfunction. Alkaline phophatase and lactate dehydrogenase activities in the plasma of the test were within range for the test fish (Selamoglu *et al*., 2012). *C. sativa* has resulted in a significant decrease and an increase in these enzymes activities in the test during the period of study. According to Wright and Plummer (1974), ALP is employed to assess the integrity of plasma membrane and endoplasmic reticulum. Therefore, the significant decrease in ALP activity revealed that the plant extract has effect on plasma membrane of the test fish; showing a significant decrease in liver ALP activity. The decrease in liver ALP activity may be responsible for the decrease in protein levels in the test fish. As Pilo *et al.* (1972) reported that the decrease in ALP activity plays an important role in protein synthesis. In this investigation, the decrease in LDH activity indicated decrease metabolic activities of the exposed fish. The increase value of serum uric acid observed in this study could be due to the liver's ability to convert excess protein by way of deamination into less poisonous urea. This agrees with work of Martinez *et al.* (2004) who stated that fish under stress fish may mobilize protein to meet energy requirement needed to sustain increase in physiological activity.

In conclusion, the study revealed that the sublethal concentrations of crude leaf extracts of *Cannabis sativa* has effects on some biochemical parameters of common carp- *Cyprinus carpio* with the fish exposed to 30.00 mg/l showing more alterations in the determined biochemical parameters.

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