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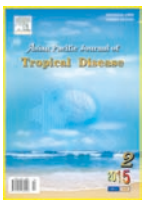

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Enzymatic and biochemical changes in common carp, *Cyprinus carpio* (L.) fingerlings exposed to crude leaf extract of *Cannabis sativa* (L.)

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PEER REVIEW

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Comments

Some active ingredients of *C. sativa* have been noted to affect the behaviour and physiology of fish. Furthermore, *C. sativa* has been reported to be an immunostimulant and has neurotoxic properties. In this present study, the author provides the active ingredients and its effects on studied fish.
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ABSTRACT

Objective: To investigate the effects of different concentrations of leaf extract of *Cannabis sativa* (*C. sativa*) on the biochemical changes and level of alterations in the enzymatic activities in serum, liver and gill of common carp, *Cyprinus carpio* fingerlings.
Methods: The fish specimens were exposed to sublethal concentrations (1.88, 3.75, 7.50, 15.00 and 30.00 mg/L) of crude leaf extract of *C. sativa* over a period of 56 d in a static renewable bioassay. The biomarkers studied were alkaline phosphatase, lactate dehydrogenase and total protein in the gill, liver and serum while aspartate aminotransferase, alanine aminotransferase bilirubin, total bilirubin, albumin, urea acid and cholesterol were determined in the serum only.
Results: Statistical significant differences ($P<0.05$) were found in all the biochemical parameters of the gills, liver and serum in all the experimental fish exposed to *C. sativa* compared with the controls. Phytochemical analysis of the leaves of *C. sativa* revealed the presence of alkaloids, flavonoids, cardiac glycosides, resins, terpins and steroids.
Conclusions: The investigation revealed that prolonged exposure of *Cyprinus carpio* fingerlings to crude leaf extract of *C. sativa* cumulated to stressful conditions in the fish. Further investigation should be conducted on the molecular mechanisms of these parameters on the biochemical and enzymatic activities as their mode operations are not well studied in fish.

KEYWORDS

Cannabis sativa, Biochemical, Enzymes, Phytochemical analysis, *Cyprinus carpio*

1. Introduction

Cannabis sativa (*C. sativa*) is a cosmopolitan weedy plant that is grown in many parts of the world. *Cannabis* contains compounds with active ingredients of varying potencies such as tetrahydrocannabinol, phytocannabinoids and plant steroids[1,2]. Studies have noted the potency of *Cannabis* as pest repellent and pesticides. The addition of *C. sativa* to the soil as organic amendment can work as nematicides and can be successfully used for controlling root-knot nematodes (*Meloidogyne incognita*) by reducing their infection and reproduction[3]; has also killed or prevented insects, mite, fungal and bacterial pathogens[4]. More so, the root exudates of *Cannabis* repelled underground larvae of the European chafer *Melolontha melolontha*[5]. Investigation provides evidence that hempseed and hempseed oil can safely be utilized as feed ingredients for laying hens to produce table eggs that are enriched in essential fatty

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acids[6]. In addition, cannabinoid intake is reported to affect mood and is associated with impaired function of a variety of cognitive tasks and short-term memory, including driving or operation of intricate machinery, muscle relaxation, immunosuppression and stimulation of appetite[7-9]. Some active ingredients of *C. sativa* have been noted to affect the behaviour and physiology of fish. Cardiac glucosides and alkaloids were reported to cause reduction in the blood glucose level of rat exposed to leaf extracts of *Vernonia amygdalina*, while terpenes caused erratic pattern of swimming in fishes[10,11]. The presence of these compounds and products in fish systems interfere with food utilization and affect the general health of fish[12].

Biochemical markers are measurable responses to the exposure of an organism to xenobiotics as well as very good biosensors of aquatic contaminants[13,14]. Aquatic pollution can easily be detected through biomarkers, as enzymes provide one of the most reliable means of assessing the degree of exposure of animals to pollutants[15,16].

Several specific enzymes such as carboxyesterase, lactate dehydrogenase (LDH), alanine and aspartate aminotransferases (ALT and AST) as well as alkaline (ALP) and acid phosphatases have been proposed for monitoring water pollution and are considered as useful biomarkers to determine pollution levels in aquatic systems[17].

Fish are ideal sentinels for detecting and documenting aquatic pollutants and largely used as bio-indicators of environmental pollution, due to their ability to retain different dissolved xenobiotics that build up in the food chains consequently resulting to adverse effects and death in the aquatic systems. Hence, there is the need to determine the mechanism of pollutant action and possible ways to mitigate their adverse effects[18-21].

The common carp [*Cyprinus carpio* (*C. carpio*)] belongs to the family Cyprinidae and is one of the most important breeder species of fish in farm culture worldwide, especially in Asia[22,23].

C. sativa have various applications with their attendant side effects, however, there is no scientific documentation of effects of leaf extract on the biochemical changes and enzymes activities in *C. carpio* especially under sub chronic exposure. Therefore, this study was undertaken to evaluate the sublethal effects of crude leaf extract of *C. sativa* on the biochemical changes and some enzymatic activities in the liver, gill and plasma of *C. carpio* fingerlings.

2. Materials and methods

2.1. Procurement and phytochemical analysis of experimental plant

A total weight of 500 g of marijuana (*C. sativa* L.) was obtained from the National Drug Law Enforcement Agency (NDLEA), Jos Plateau State, Nigeria, command strictly for scientific research. The leaves (405 g) were carefully sorted out from the stem/twigs by handpicking and then separately powdered (398.86 g) using mortar and pestle and sieved through a metal sieve (90 μm mesh size) and stored in airtight polyethylene bags.

The phytochemical analysis was conducted to determine the alkaloid, flavonoids, tannins, phenols, cardiac glucosides, terpenes and steroids, resins volatile oils, and balsam present in crude leaf extract of *C. sativa*. A stock solution of the crude leaf extracts was prepared for the phytochemical analysis by macerating 5 g of the dried powdered leaves in 200 mL of petroleum ether for 24 h at 25 °C. This resulted to 300 g weight of oily resin after evaporating the solvent at room temperature (26±1) °C. To this weight (300 g), 25 mL of acetone was used to dissolve the resin resulting to a 12 mg/mL solution. From this concentration (12 mg/mL), quantities were drawn for phytochemical analysis as described by previous studies[24,25]. The remaining part of leaf extract was used for *in vivo* exposure experiment.

2.2. Collection of experimental fish and acute toxicity test

Two hundred fingerlings of *C. carpio* with average weight of (15.05±0.05) g 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 oxygenated polyethylene bags and were held in concrete rectangular tanks (3.0 m×1.5 m) and allowed to acclimatized for two weeks. During the acclimation period and sublethal exposure, the fish were fed to satiation with 3 mm commercially pelleted fish feed (Multifeeds®; protein 42%, fat 12%, ash 7.5% fiber 2.6%). Dechlorinated tap water was used and the entire quantity was changed after seven days, during which mortality recorded was less than 1%. Photoperiod in the experimental environment was natural (14 D: 10 L) with ambient temperature of (26±1) °C and average water temperature of (22.40±0.05) °C. Prophylactic or therapeutic treatments were not administered because fish were apparently healthy and active[26].

A stock solution of the crude leaf extract of *C. sativa* for the acute toxicity test was prepared by macerating 12.5 g of the dried powdered leaves of *C. sativa* in 500 mL of petroleum ether for 24 h at 25 °C. This resulted to an oily (950 mg petroleum ether free) resin. From this resin weight (950 mg), five different concentrations of 60, 120, 180, 240 and 300 mg were prepared in duplicate. The solutions were obtained by serial dilution of the stock with acetone. Two other groups were used as contrast groups, one group was exposed to tap water and acetone group was exposed to acetone (v/v, 0.05%) used for the highest leaf

extract concentration. A total of 10 fish were distributed in each test concentration and controls in (60 cm×40 cm×40 cm) aquaria. The experiment was conducted according to the methods described before[27]. The tests were performed in a semi-static assay for 96 h without feeding. The test water was renewed daily to maintain the concentration of the extract and water quality. Furthermore, ethical guidelines on animals in Department of Zoology, University of Jos, were strictly followed in carrying out the experiment.

2.3. Determination of sub-lethal concentrations and in vivo exposure experiment

The 96 h LC_{50} of leaf extract of *C. sativa* in the present research based on the probit analysis was estimated to be 90.00 mg/L. From the value of 96 h LC_{50} obtained, five sub-lethal concentrations of 30.00 (1/3 of LC_{50}), 15.00 (1/6 of LC_{50}), 7.50 (1/12 of LC_{50}), 3.75 (1/24 of LC_{50}) and 1.88 mg/L (1/48 of LC_{50}) and two controls were prepared in duplicate. Two other groups were used as contrast groups, one group exposed to tap water and acetone group to the volume of acetone (v/v, 0.05%) used for the highest leaf extract concentration. A total of 10 fish were distributed in each test concentration and controls in (60 cm×40 cm×40 cm) aquaria. The fish were kept for a exposure period of 8 weeks (56 d). The experimental aquaria were supplied with continuous dissolved oxygen through a giant aeration pump. The test solutions were renewed regularly to maintain the concentration of the test extract. Physico-chemical parameters were measured throughout the 56-day trial by the previous methods[28]. At the end of 56-day exposure period, the serum, gills, and liver of exposed and control fish were examined for 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) to ascertain the level of biochemical and enzymatic alterations.

2.4. Biochemical and enzyme assay

At the end of the 56-day exposure to sub-lethal concentrations of *C. sativa* crude leaf extract, fish from each replicate were subjected to blood collection (1 mL) through cardiac puncture using non heparinised syringe and needle. Blood obtained was immediately centrifuged at 1 000 r/min for 5 min to obtain the plasma serum. The activities of ALP, LDH, TP, AST, ALT, BIL, TBIL, UA and CHOL were assayed using GENESYS-20 spectrophotometer (GENESYS 20, Thermo Electron Corporation, USA). Similarly, tissue samples of test and control fish were rapidly taken, three each from replicate, washed in normal saline to remove excess blood and immediately stored in sample bottles in ice packed cooler (0 °C) until analysis. The gills and liver samples were homogenized in buffer (0.25 mol/L sucrose, 0.01 mol/L TRIS and 0.01 mol/L EDTA) and centrifuged at 1 000 r/min for 10 min. The supernatant was decanted and introduced into cuvette tube for spectrometric absorbance measurement at different wave lengths using commercially prepared reagents kits (Randox; Spectrum; Fortress). The biochemical and enzymatic activities were determined following the methods: ALP, LDH, AST and ALT[29], TP[30], BIL and TBIL[31], UA and CHOL[32,33].

2.5. Statistical analysis

Data obtained were analyzed using the statistical package SPSS 17.0 computer program (SPSS Inc. Chicago, Illinois, USA). Differences in the parameters between applied test concentrations and durations were subjected to One-way analysis of variance (ANOVA) followed by Duncan's multiple range tests to determine significance difference at 5% level of probability.

3. Results

3.1. Phytochemical analysis

The results of the phytochemical screening of *C. sativa* leaf showed that the Dragendoff's reagent test produced orange coloration confirming the presence of alkaloids. There was no visible change in the appearance of the leaf extracts after conducting tests for the presence of saponins, tannins, balsam and volatile oils implying that these phytochemicals are not present in the leaves of *C. sativa*. The presence of light yellow, reddish brown, violet, reddish brown color at the interphase between acetic acid anhydride layer and sulphuric acid layer confirmed the presence of flavonoids, cardiac glycosides, resins, terpenes and steroids respectively in the leaf extract of *C. sativa* (Table 1).

Table 1
Qualitative phytochemical screening of crude leaf extract of *C. sativa*.

Phytochemical	Quality	Color	Test
Alkaloid	+++	Orange	Dragendoff's
Saponin	−		
Flavonoids	++	Light yellow	Lead acetate
Tannins	−		
Cardiac glycosides	+++	Reddish brown	Keller-Killani
Balsam	−		
Phenols	−		
Terpenes & steroids	+++	Reddish brown	Burchard
Resins	+++	Violet	
Volatile oils	−		

+++: high presence; ++: moderate presence; −: absence.

3.2. Water quality parameters

The mean water quality parameters measured during the 56 d exposure of *C. carpio* fingerlings to sub lethal concentrations of crude leaf extracts of *C. sativa* are presented in Table 2. There was no significant difference ($P<0.05$) in the temperature of all the test tanks including the control groups as temperature was uniform (23 °C) in all the test tanks

Table 2

Water quality parameters of the sublethal concentrations of *C. sativa* crude leaf extract on *C. carpio* fingerlings for 56 d (mean±SE).

Parameters	Concentrations (mg/L)						
	30.00	15.00	7.50	3.75	1.88	Control Acetone	Control Water
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	6.20±0.45	6.20±0.45	6.40±0.41	6.60±0.22	6.60±0.31	7.00±0.00	7.00±0.00
Dissolved oxygen (mg/L)	4.00±0.22	4.10±0.22	4.10±0.22	4.10±0.22	4.50±0.00	4.50±0.00	4.50±0.00
NH_3 (mg/L)	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00
Alkalinity (mg/L)	36.60±3.67	35.48±3.15	34.02±2.03	32.87±1.68	32.38±1.54	30.31±0.27	30.25±0.49
NO_3-N (mg/L)	0.13±0.04	0.13±0.04	0.11±0.04	0.10±0.03	0.70±0.04	0.70±0.04	0.09±0.04
CO_2 (mg/L)	5.18±0.48	4.93±0.43	4.68±0.22	4.49±0.31	4.41±0.22	4.31±0.18	4.33±0.16

including the controls. However, pH showed a decreasing trend with increase in the concentration of the test material and ranged between 6.75 and 6.92. Carbon (IV) oxide showed an increasing pattern with increase in the concentration of the crude extract with a range of 4.23 to 5.89 mg/L. Dissolved oxygen content decreased proportionately with increase in the concentration of the leaf extract in all the experimental tanks with values in the range of 4.23 to 5.56 mg/L while the control tank recorded a high value of 6.66 mg/L.

3.3. Biochemical and enzymatic alterations

The activities of enzymes, liver and renal function tests of fish in the control and sublethal test concentrations differ significantly ($P<0.05$) and are presented in Figures 1-6. The activities of ALP in the gills, liver and serum decreased linearly with increase in test concentration (Figure 1). The gills highest ALP (221.61 IU/L) was recorded in control-water (0.00 mg/L) tank while the lowest value of 121.26 IU/L was recorded in 30 mg/L. Blood LDH activities assayed for the same tissues were observed to increase with increase in the sub lethal concentrations of *C. sativa* which differ significantly ($P<0.05$) from those of the control groups; test fish at 30 mg/L recorded the highest LDH activities of 93.31, 88.43 and 113.52 IU/L in the gills, liver and serum respectively (Figure 2). Similar trend was obtained in this study for the activities of AST and ALT in the serum of the test fish which differ significantly ($P<0.05$) from those of the control groups. Highest serum AST (38.33 IU/L) was obtained in 15 mg/L while ALT had the highest concentration value of 35.65 IU/L at 30.00 mg/L. (Figure 3).

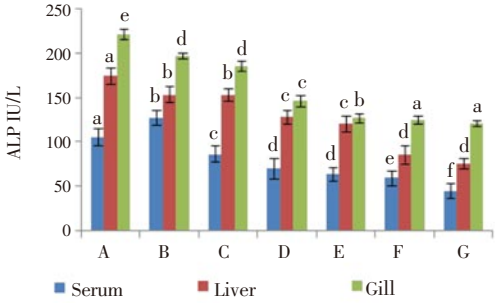

Figure 1. Changes in ALP in gills, liver and serum of *C. carpio* fingerlings exposed to controls and different concentrations of crude leaf extract of *C. sativa* for 56 d.

Different letters indicate significant difference ($P<0.05$) in mean values among concentrations and controls. A: Control water; B: Control acetone; C: 1.88 mg/L; D: 3.75 mg/L; E: 7.50 mg/L; F: 15.00 mg/L; G: 30.00 mg/L.

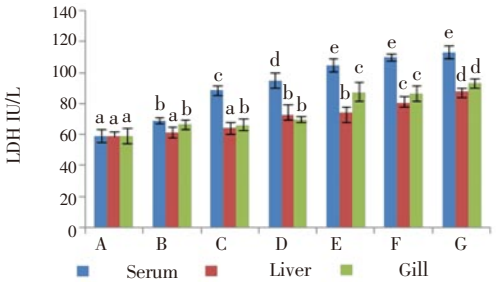

Figure 2. Changes in LDH in the gills, liver and serum of *C. carpio* fingerlings exposed to controls and different concentrations of crude leaf extract of *C. sativa* for 56 d.

Different letters indicate significant difference ($P<0.05$) in mean values among concentrations and controls. A: Control water; B: Control acetone; C: 1.88 mg/L; D: 3.75 mg/L; E: 7.50 mg/L; F: 15.00 mg/L; G: 30.00 mg/L.

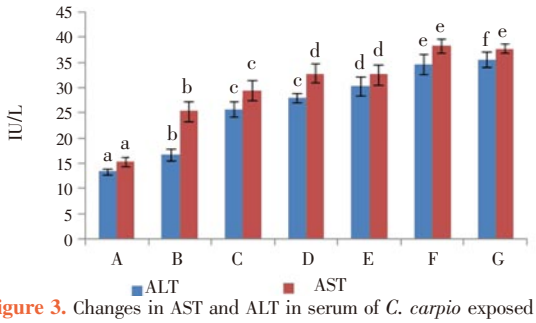

Figure 3. Changes in AST and ALT in serum of *C. carpio* exposed to controls and different concentrations of crude leaf extract of *C. sativa* for 56 d.

Different letters indicate significant difference ($P<0.05$) in mean values among concentration and controls. A: Control water; B: Control acetone; C: 1.88 mg/L; D: 3.75 mg/L; E: 7.50 mg/L; F: 15.00 mg/L; G: 30.00 mg/L.

TP in the gills, liver and serum exhibited a slightly different pattern of activities (Figure 4). TP concentrations decreased with increase in concentration in gill and serum while liver showed an increase in concentration of the extract which differ significantly ($P<0.05$) from the control groups; elevated TP values of 18.81 and 15.96 IU/L were obtained in gills and serum at 0.00 mg/L control water and control acetone respectively while liver had highest TP value of 15.58 IU/L at 30 mg/L (Figure 4). Renal (kidney) function test for serum UA activity increased with increase in the sublethal concentrations of *C. sativa*; the highest UA value (136.39 g/dL) was recorded in test concentration of 30 mg/L (Figure 5). There was significant difference ($P<0.05$) in the UA activities of the fish exposed to sublethal concentrations of *C. sativa* compared with those of the control groups. Significant difference ($P<0.05$) was also obtained in the CHOL activity of the tests fish from those of the control tanks. CHOL activity decreased with increase in the test material concentration.

The highest CHOL concentration of 182.87 g/dL was recorded in 0.00 mg/L (control-water) test concentration while the lowest value of 112.52 g/dL was recorded in 30 mg/L (Figure 5). Liver function tests for serum ALB, TBIL and BIL all increased with increase in the concentration of the test material and differ significantly ($P<0.05$) from the control groups (Figure 6). The hypercholesterolemia observed in the control groups could suggest the potentials of *C. sativa* to reduce tissue cholesterol concentrations. There were significant differences ($P<0.05$) in the activities of serum ALB, TBIL and BIL concentrations in test fish serum compared to the control groups; elevated levels of ALB (1.52 mg/dL), TBIL (0.86 mg/dL) and BIL (1.07 mg/dL) were recorded at 30.00 mg/L while the control-water group, recorded 1.18, 0.71 and 0.94 mg/dL respectively (Figure 6).

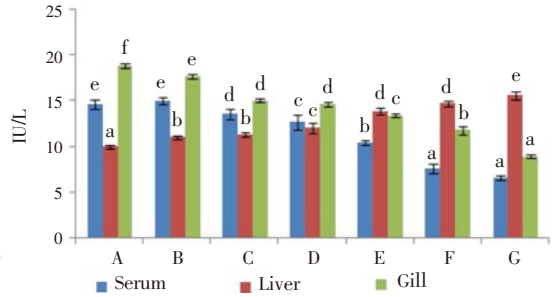

Figure 4. Changes in TP concentrations in the gills, liver and serum of *C. carpio* fingerlings exposed to controls and different concentrations of crude leaf extract of *C. sativa* for 56 d.

Different letters indicate significant difference ($P<0.05$) in mean values among concentrations and controls. A: Control water; B: Control acetone; C: 1.88 mg/L; D: 3.75 mg/L; E: 7.50 mg/L; F: 15.00 mg/L; G: 30.00 mg/L.

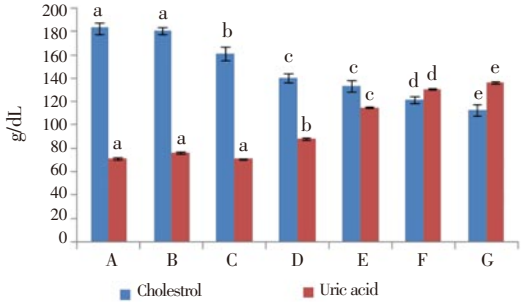

Figure 5. Changes in cholesterol and uric acid activities in the Serum of *C. carpio* fingerlings exposed to controls and different concentrations of crude leaf extract of *C. sativa* for 56 d.

Different letters indicate significant difference ($P<0.05$) in mean values among concentrations and controls. A: Control water; B: Control acetone; C: 1.88 mg/L; D: 3.75 mg/L; E: 7.50 mg/L; F: 15.00 mg/L; G: 30.00 mg/L.

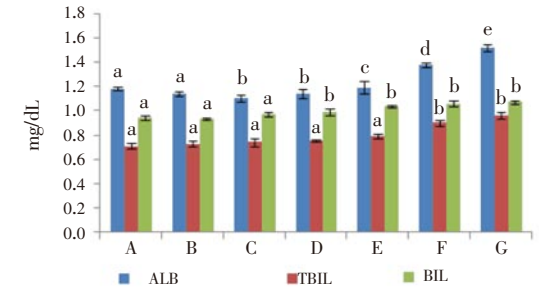

Figure 6. Changes in ALB, TBIL and BIL in the serum of *C. carpio* fingerlings exposed to controls and different concentrations of crude leaf extract of *C. sativa* for 56 d.

Different letters indicate significant difference ($P<0.05$) in mean values among concentrations. A: Control water; B: Control acetone; C: 1.88 mg/L; D: 3.75 mg/L; E: 7.50 mg/L; F: 15.00 mg/L; G: 30.00 mg/L.

4. Discussion

Water quality parameters obtained in this study were found to be within acceptable range for fish culture as suggested by previous study[34]. Dissolved oxygen value of the test media significantly ($P<0.05$) decreased with increase in concentration of the test material but did not exacerbate measurable stress in the fish exposed to the sublethal concentrations of *C. sativa*. Temperature was within acceptable limits for fish culture and did not differ significantly ($P>0.05$) in all the test tanks including the controls[34]. There was decrease in pH with increase in sublethal concentration of *C. sativa*; however, neutral pH values were recorded in the control groups which differ significantly ($P<0.05$) from those of the test media. Alkalinity values differ significantly ($P<0.05$) from those of the control groups and increased linearly with increase in toxicant concentration which might have neutralized the change in pH with increase in sublethal concentrations of *C. sativa*. This could probable explain the 100% survival rate recorded in all the experimental tanks. This view is supported by the previous work[35], which reported that alkalinity above 20 mg/L can significantly increase the survival of fishes.

Phytochemical screening of *C. sativa* leaves revealed the presence of alkaloids, flavonoids, cardiac glucosides, terpenes, resins and steroids. These compounds when present in animal's body have been reported to have toxicological consequences in the animal[36,37]. Cardiac glucosides and alkaloids were reported to cause reduction in the blood glucose level of rat exposed to leaf extracts of *Vernonia amygdalina*, while terpenes caused erratic pattern of swimming in fishes[10,11]. The presence of these compounds and their by products in fish systems interfere with food utilization and affect the general health of fish[12]. Although, saponin is diversely distributed among the plant family, this compound was not found in the leaf extracts of *C. sativa*.

During the 56 d of maintaining *C. carpio* fingerlings in different sublethal concentrations of *C. sativa* crude leaf extract, significant differences were noticed in all the plasma biochemical parameters of ALP, LDH, AST and ALT; same went for the liver and renal functions of TP, ALB, BIL, TBIL, UA; and CHOL compared to fish in the control groups. This could be attributed to the assertions in previous work[38], that toxicants cause disturbances in the physiological states of animals which inadvertently affect enzymes activities resulting to elevation or inhibition in activities of the enzymes. In line with this assertion[39], Pourgholam *et al.* reported significant and insignificant increase or decrease in some biochemical parameters of *Ctenopharyngodon idella* such as AST, ALT, ALP and LDH exposed to sublethal concentrations of diazinon. Generally, biochemical and haematological fluctuations in fishes are the first discovered

and measured responses caused by environmental changes, which can give important information about the inwards of organisms[40]. Alkaline phosphatase is a membrane bound glyco protein enzyme found at bile pole of hepatocytes, pinocytic vesicles, golgi complexes and cell membranes, with a high concentration in sinusoid and endothelium where they are involved in active transport across membranes as hydrolases or transphosphorylases and often employed to access the integrity of plasma membrane[41,42]. In this study, ALP levels in the blood, gills and liver decreased significantly ($P<0.05$) with increase in sublethal concentrations of *C. sativa*. Similar decrease in plasma ALP were also reported by other authors when they exposed fishes to various toxicants concentrations[20,41]. The decrease in ALP activities may be due to the damage of hepatic tissues with disturbed normal liver function. This view was reported by Gabriel UU *et al*.[43], who concluded that decrease in ALP activity may be taken as an index of hepaticytic parenchyma damage and necrosis shows an interruption in the secretion and flow of bile in the liver. Contrary, other authors reported elevation in ALP activities in fishes exposed to various toxicant concentrations[44,45].

Activities of LDH in serum, gills and liver increased significantly ($P<0.05$) with increase in sublethal concentrations of test material compared with those of the control groups. This observation was also reported when *C. gariepinus* fingerlings were exposed to crude oil and sublethal concentrations of potassium permanganate[46,38]. The increase in LDH across the experimental groups observed in our study could be attributed to necrosis of the liver hepatocytes; a view shared by Vasudevan DM *et al*.[47], and not necessarily due to an increase in anaerobic carbohydrate metabolism[38], since the water quality parameters of the tests tanks remained within acceptable limits. In this investigation, the decrease in LDH activity indicated decrease metabolic activities of the exposed fish. The inhibition of these enzymes would result in the accumulation of metabolic intermediates in the liver which could cause physiological stress in fish[18,48].

Transaminases are biomarker enzymes endogenous in the liver responsible for transformation of proteins to glycogen[49]. Data obtained in this study for the activities of AST and ALT showed that their activities increased significantly ($P<0.05$) from those of the control groups in accordance with the previous works[50-52], and an indication of liver damage[53,54]. In a related study[55], it noted significant increase in the activity of plasma AST and ALT when rats were injected with *C. sativa*. In like manner[56], it was showed that increase in cassava mill effluent exposed to adult *C. gariepinus* aggravated high levels of AST and ALT activities which are suggestive of hepatic cellular damage leading to their leakage into circulation. It is generally accepted that an increase in these enzymes activities in the extracellular fluid or plasma is a sensitive indicator of even minor cellular damage[53,57].

Total protein plays a vital role in the physiology of living organisms[58]. The most portion of serum protein synthesis is dependent on the liver and it can be used as an indicator of liver dysfunction. Reduction of total protein concentration is an obvious feature of many diseases such as liver disease, the absorption of, reduction or loss of protein[59]. In the present study exposure to leaf extract of *C. sativa* resulted in significant decrease in total protein levels in the blood of *C. gariepinus*. The decrease may be attributed to the utilization of protein as an energy source to compensate for increased energy demand to cope with leaf extract-induced stress[60]. Further more, hypoproteinaemia was evident in all experimental groups, and agreed with reports of several authors[13,60-63].

The activities of TBIL and BIL observed in this study did not reveal any defined pattern with increase in sublethal concentration of *C. sativa* over 56 d. There were fluctuations in the activities of these liver function indices; with high level of total bilirubin attributed to damage to hepatocytes which is evident in the histopathology results we obtained or could be due to resultant red blood cells haemolysis as suggested[64]. 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 piosonous urea. Our conclusion is that, the observed significant ($P<0.05$) increase in urea concentration was as a result of increase in total protein concentration in the liver with increase in sublethal concentrations of crude leaf extracts of *C. sativa*. This agrees with work of Martinez CBR *et al*.[65], who stated that fish under stress may mobilize protein to meet energy requirement needed to sustain increase in physiological activity. However, disagrees with the report of Dogan D and Can C[60], who asserted that there was decrease in uric acid activity with increase in toxicant concentration due to the inability of the liver to synthesize purine and that hypouricaemia an abnormal decrease in uric acid is usually associated with hepatocellular disease and renal reabsorption defect.

Cholesterol which measures the food status in animals has been shown to increase in toxicant concentration due to damage to the liver or kidney[22]. This is however at variance with our observed hypocholesterolemia with increase in sublethal concentration of *C. sativa* which agreed with the finding[45]; besides, the observed histological deformities of the liver sections did not cumulate to death of the test fish. An important note asserted that cholesterol is the most important sterol occurring in plasma and red blood cells[20]. If this assertion be true then, it is logical to add that with decrease in red blood cells content due to increased sublethal concentrations of *C. sativa*, cholesterol concentrations in the blood of the exposed fish should

similarly decrease in concentration. The implications of these findings are that crude leaf extracts of *C. sativa* is biochemically important in interfering with normal physiological processes of *C. carpio* fingerlings.

In conclusion, the result of this investigation indicates that exposure of crude leaf extracts of *C. sativa* to common carp; *C. carpio* has tremendous effects on the biochemical and enzymatic activities thereby affecting the general physiology of the fish. The parameters analysed could be good biomarkers of exposure to sublethal concentration of the leaf extracts. Further investigation should be conducted on the molecular mechanisms of these parameters on the biochemical and enzymatic activities as their mode operations are not very studied in fish.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

C. sativa has various applications which includes; potential for inclusion in animal feeds requirement, ameliorating stress conditions during fish handling, sampling and transportation as well as possible utilization to anesthetize fish. Nevertheless, studies have implicated *C. sativa* with toxicological consequences. Researchers have noted the toxic effects of *C. sativa* on the animals such as rat, mice, dog, cattle including human. Their investigations included severe anxiety and panics, paranoia and psychosis as well as hallucinations. However, the toxicological implications of *C. sativa* on fish are not documented, especially on the biochemical and enzymatic activities in the common carp, *C. carpio* under chronic exposure.

Therefore, this study was undertaken to evaluate the sublethal effects of crude leaf extract of *C. sativa* on the biochemical changes and some enzymatic activities in the liver, gill and plasma of *C. carpio* fingerlings.

Research frontiers

The implications of these findings are that crude leaf extracts of *C. sativa* is biochemically important in interfering with normal physiological processes of *C. carpio* fingerlings. The parameters analyzed could be good biomarkers of exposure to sublethal concentration of the leaf extracts especially in *C. carpio*.

Related reports

In this present study, the authors followed standard procedures to perform the photochemical screening of the *C. sativa* as well as the toxicological studies. The results suggest that the phytochemical properties of the leaf extract may have contributed to the changes in the various parameters investigated.

Innovations & breakthroughs

To our knowledge, there is no work enzymatic and biochemical changes in common carp, *C. carpio* fingerlings exposed to crude leaf extract of Marijuana (*C. sativa*). The present investigation provides first hand information on biomarkers studied

Applications

C. sativa has potential for inclusion in animal feeds requirement, ameliorating stress conditions during fish handling, sampling and transportation as well as possible utilization to anesthetize fish. However, it can also be used in toxicological studies especially in fish.

Peer review

Some active ingredients of *C. sativa* have been noted to affect the behaviour and physiology of fish. Furthermore, *C. sativa* has been reported to be an immunostimulant and has neurotoxic properties. In this present study, the author provides the active ingredients and its effects on studied fish.

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