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BIOACCUMULATION AND CLEARANCE OF CURCUMIN FROM GILL, LIVER, KIDNEY, AND MUSCLE OF FRESHWATER CATFISH, *HETEROPNEUSTES FOSSILIS*

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ABSTRACT
Heteropneustes fossilis is a stinging catfish with excellent market demand because of its low spine, fat content, high protein, calcium, and many essential nutrients. Turmeric has a significant role in preserving the nutritional content of aquaculture food products, and prolonging the shelf life because of its antibacterial and antioxidant properties. It has a high affinity for lipids but low availability in water, which restricts its benefits. In the current study, adult *H. fossilis* were treated with curcumin for a short duration (1/5 d) to a long period (21 d), along with a post-treatment clearance group (one-week of incubation without curcumin after 21 d of treatment). We checked the bioaccumulation of curcumin in vital tissues (liver, kidney, gill, and muscle) of freshwater catfish and the rate of clearance from the body system. The findings revealed that an effective concentration of curcumin (10 µg/mL) in the medium did not cause significant bioaccumulation in fish tissue (gills, liver, kidneys, or muscles) for up to 21 d on the treatment plane and was fully cleared from the freshwater catfish biosystem. This study further strengthens the understanding of the mechanism of action of curcumin in pharmaceuticals at the local level and in a regular effective dose-dependent manner.

Keywords: *Curcumin, Bioaccumulation, Heteropneustes fossilis, Vital tissue, Biological clearance*

INTRODUCTION
Fish are an essential source of protein for human nutrition in aquaculture. Fisheries resources are important in many aspects of the national economy, including nutrition, culture, employment, foreign exchange profits, traditional dietary habits, and others (Lovell, 1997). *Heteropneustes fossilis*, is also known as Asian stinging catfish or singhi. It is very nutritious, appetizing, and flavourful, and is widely preferred in many regions of the Indian subcontinent because of its low cholesterol, low fat, and high digestion (Khan *et al.,* 2003). In comparison to many other freshwater fishes, this species has a relatively high iron concentration (226 mg/100 g) and a moderately high calcium level (Saha and Guha, 1939; Alok *et al.*, 1993). Food safety has become increasingly important as the significance of expanding food production and processing, preserving food quality, preventing nutritional losses, and prolonging shelf life has increased. As a result, using food additives is the most effective approach for increasing aquaculture output and safety.

Turmeric is a commonly consumed ingredient made from the root of the *Curcuma longa* plant, a ginger family fibre plant. Turmeric, the powdered dried rhizome of the plant *Curcuma longa*, is extensively used as a colourant and spice in a variety of foods (Rahman *et al.*, 1982) Curcumin (diferuloylmethane; 1, 7-bis [4-hydroxy-3-methoxyphenyl]-1,6-heptadiene-3,5-dione) is the main bioactive ingredient of turmeric. Curcumin (75%), demethoxycurcumin (15%), and bisdemethoxycurcumin (5%) are the three curcuminoids found in commercially available natural curcumin (Priyadarsini, 2014). The attractiveness of aquaculture food can be increased by adding colour, and taste and ensuring healthy products. It triggers cell signalling proteins, influencing many molecular targets, cell cycle proteins, chemokines, cytokine enzymes, receptors, and cell surface adhesion molecules, all of which have been shown to be beneficial in several studies (Joe *et al.*, 2004; Shishodia *et al.*, 2005). Because of these characteristics, they may be obtained as additives in food preparation and have a favourable effect on consumer metabolism. Despite its effectiveness and safety, the therapeutic potential of curcumin is still debatable, owing to its low absorption in humans, even when it is given at high doses (12 g/d) (Ataie *et al.*, 2010). Curcumin has limited oral bioavailability due to small intestine absorption combined with substantial reductive and conjugative metabolism in the liver and excretion through the gall bladder. Its low bioavailability is exacerbated by its binding to enterocyte proteins, which can alter its structure (Ringman *et al.*, 2005). It is hydrophobic and readily dissolves in acetone, dimethylsulfoxide, oils, and ethanol. A review of studies revealed that several analytical procedures, including UV-visible, HPLC, and HPTLC, have been developed for their analysis, but only for plasma and urine (Anand *et al.*, 2007; Akazawa *et al.*, 2012) and not for fish tissue.

In this study, we measured curcumin spectrophotometrically in catfish tissues (liver, kidney, and gill) and muscle to determine whether it shows bioaccumulates or tends to be cleared even when it is applied under *in vivo* conditions (for maximum pharmaceutical application).

MATERIALS AND METHODS

Chemicals

Curcumin (CAS No. 458-37-7, $C_{21}H_{20}O_6$, 368.38-g/m, purity 97.00% with HPLC; Himedia) and other chemicals used in the study were of analytical grade and were purchased locally from scientific suppliers, Lucknow.

Animal collection and acclimatization

Adult *Heteropneustes fossilis* (25 - 30 g) were collected from the local fish supplier. They were acclimatized under a natural photoperiod and temperature (12 h dark: 12 h light) in laboratory conditions. They were given food (goat liver) twice a day. The water was replaced daily to avoid the accumulation of excreta and unconsumed food.

Experimental setup

Experiments were conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA, 2021) so that there would not be any kind of cruelty to the fish.

The stock solution of curcumin was prepared in ethanol and further working solutions were made in aquarium water.

Adult catfish *H. fossilis* were incubated for 24 h in media supplemented with different concentrations (0.1-100 µg/mL) of curcumin (five fish in each concentration) along with control media (with water only) to determine the effective concentration based on their haematological parameter analysis (data not shown). The effective concentration of curcumin (10 µg/mL) was evaluated for the duration study (1/5/21 d) to examine the effect of duration on its bioaccumulation in important vital tissues (liver, kidney, gill, and muscle) in the second experimental setup. To quantify the amount of curcumin that was eliminated from the fish muscle and essential tissue, the fish were maintained in freshwater for 7 d after a 21 d treatment period in the third experimental setup. The media was changed with a fresh dose of chemical every 24 h to maintain chemical constituents and to avoid any accumulation of metabolic waste. The experiments were performed in duplicate with five fish in each set.

The fish from each group were sacrificed at the end of the experiment, and the tissues (muscles, liver, kidney, and gill) were collected and stored at -20^0C for estimation of the curcumin concentration.

Sample preparation

The determination of curcumin pigmentation was performed (Hanif *et al.*, 1997). In brief, we weighed 100 mg of fish tissue organs (muscles, kidney, liver, and gill). The mixture was sonicated for 30 min to a volume of 100 mL. The resultant solution was centrifuged for 15 min at 2500 rpm, and the supernatant was tested for the presence of the curcumin pigment in duplicate. Similarly, commercial curcumin was prepared in methanol as a standard for evaluating its concentration. The standard and samples were analysed at a wavelength of 421 nm with a UV-visible spectrophotometer (Eppendorf, Germany) the Concentration was evaluated with the following formula:

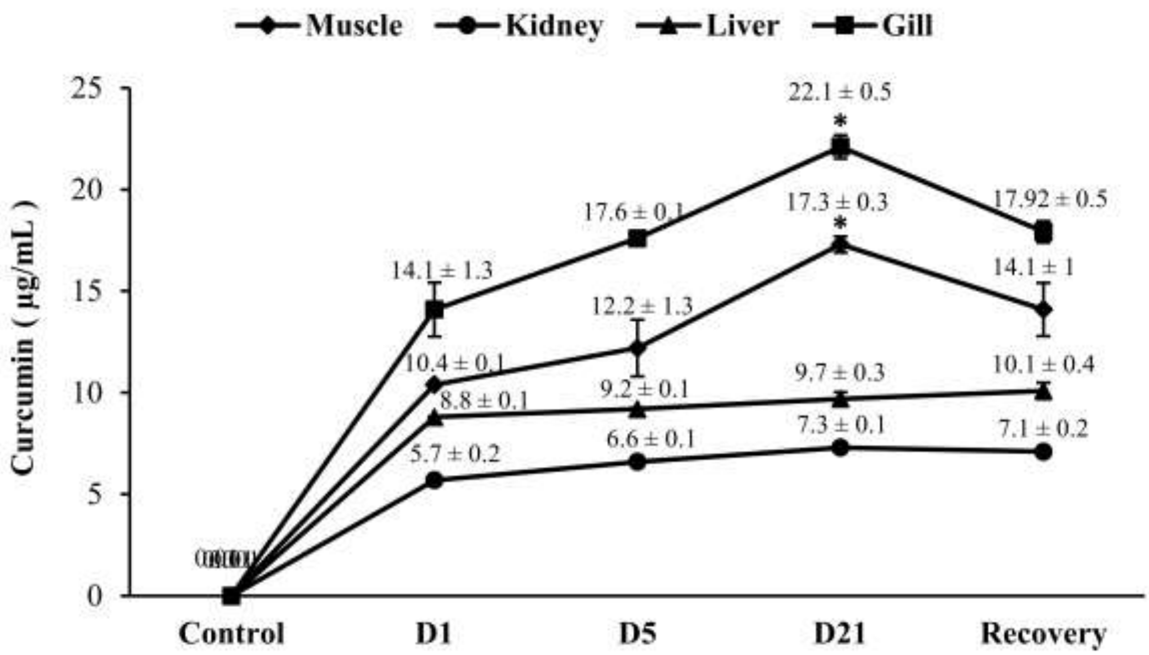
O.D. of unknown / O.D. of known X standard concentration

Statistical analysis

Data were represented as means ± SEM. The significance of values obtained from different groups was tested by using a one-way analysis of variance (ANOVA). Intergroup comparisons were performed with the Newman-keuls' test ($P < 0.05$).

RESULTS

The freshwater catfish have shown the best histology along with haematological parameters at 10 µg/mL as compared with other tested curcumin concentrations. Based on haematological data (Pandey *et al.*, 2022) the effective concentration (10 µg/mL) of curcumin for freshwater catfish, *H. fossilis*, was selected. An effective concentration of curcumin was used in the duration study (1/5/21 d) to determine its bioaccumulation in major vital organs. The results showed a significant increase in curcumin in muscle and gill tissue after 21 d of continuous curcumin treatment (Figure 1). This may be due to continuous exposure of the first gill, followed by subcutaneous muscle in the curcumin water. As the colorimetric method was the best method, tissue (gill and muscle) significantly absorbs the yellow dye of curcumin due to its lipid affinity. However, curcumin did not exhibit significant bioaccumulation in



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ner vital tissue (kidney, liver) regardless of duration (1/5/ 21 d; Figure 1).

Figure 1: Estimation of curcumin (10 µg/mL) accumulation with duration (day 1 - 21 with recovery of 7days without curcumin treatment) on freshwater catfish, *Heteropneustes fossils* in their muscle and other vital organs (liver, kidney, and gill). Values expressed as mean ± SEM. Data were analyzed by one way ANOVA (*P* < 0.001) followed by Newman-keuls' test (P* < 0.05)**

After a clearance period of one week without curcumin, there was no significant change in the amount of curcumin in vital tissue compared to that in the control group. The yellowness (curcumin dye) that we detected in the tissue after the curcumin water incubation was also cleared from the tissues within one week duration after curcumin treatment for 21 d (Table. 1). This suggests that curcumin itself does not bioaccumulate in fish body organs; it has a one-way entry route inside the body system via the alimentary canal. Curcumin enters the body, makes a positive contribution to overall health, and leaves the body without long-term storage. This mechanism makes curcumin a local biomolecule, not a biomolecule that may also have an effect in its absence.

Table 1: Estimation of curcumin accumulation with duration on freshwater catfish, *Heteropneustes fossils* in their muscle and other vital organs (liver, kidney, and gill)

	Control	D1	D5	D21	Recovery
Gill	0.01 ± 0	14.1 ± 1.3	17.6 ± 0.1	*22.1 ± 0.5	17.92 ± 0.5
Muscle	0.01 ± 0	10.4 ± 0.1	12.2 ± 1.3	*17.3 ± 0.3	14.1 ± 1
Liver	0.001 ± 0	8.8 ± 0.1	9.2 ± 0.1	9.7 ± 0.3	10.1 ± 0.4
Kidney	0.01 ± 0	5.7 ± 0.2	6.6 ± 0.1	7.3 ± 0.1	7.1 ± 0.2

Values expressed as mean ± SEM. Data were analyzed by one way ANOVA ($P < 0.001$)

A yellow hue of curcumin was observed in tissue mainly gill tissue. This was due to direct bathing in the coloured solution in which the dye was stuck to the mucus (curcumin has lipid affinity). The internal organ muscles also exhibited a yellow hue. However, compared with the control fish tissue, the treated fish tissue exhibited a typical yellow shade of curcumin dye.

DISCUSSION

The current study provides direct evidence of curcumin clearance from the biosystem of the freshwater catfish *H. fossilis*. These results support the rapid metabolism and clearance of curcumin in the body (Ravindranath and Chandrasekhara, 1980). This might be due to its ease of removal, excretion, lack of distribution, shorter retention duration, and increased metabolism in the body, all of which limit its employment in the pharmaceutical sector (Li *et al.*, 2011). Deu to its weak solubility and hydrophobic nature, curcumin did not exhibit any significant retention in the essential organs.

Curcumin and its constituents are physiologically active molecules that play important roles in herbal medicines. They have low bioavailability, fast metabolism, poor absorption, and rapid systemic clearance. This effect appears to be an important factor contributing to the low curcumin in the tissue system (Lovell, 1972). In the present study, freshwater fish did not show significant curcumin bioaccumulation after continuous curcumin treatment for a longer duration except in muscle and gill tissues. The results were further supported by one-week clearing experiments. The curcumin level was close to that of the control group, suggesting that, first curcumin has no bioaccumulation properties, and second, it is cleared from the biological system. Therefore, curcumin shows temporary, local-level biological significance but does not show prolonged sustained benefits in its absence for a long duration. It does not demonstrate bioaccumulation in fish tissues. It is stable at acidic pH in the stomach (Khan *et al.*, 2003), yet it has limited bioavailability (Rahman *et al.*, 1982). Curcumin is rapidly metabolized in the

liver, where it is glucuronidated and sulphated before being excreted (Priyadarsini, 2014). Curcumin has low bioavailability *in vivo* after oral consumption (Joe *et al.*, 2004).
Curcumin uptake and distribution in bodily tissues are essential for its metabolic activities, but only a few researchers have investigated this topic (Cao *et al.*, 2015; Li *et al.*, 2022). Studies mainly depend on the dietary mode of curcumin application, unlike the present study. No changes in the level of curcumin were detected in the liver or kidney following the oral administration of 400 mg of curcumin to rats (Ravindranath and Chandrasekhara, 1980). Curcumin was detected in the stomach and small intestine in 90% of patients after 30 min, but just 1% after 24 h (Shishodia *et al.*, 2005). When averted sacs of the rat intestine were incubated with 50–750 g of curcumin in 10 mL of incubation media, 30–80% of the curcumin disappeared from the mucosal side, and no curcumin was identified in the serous fluid, according to an *in vitro* investigation. At the maximum curcumin concentration, less than 3% of the curcumin was detected in the tissues. Another study used tritium-labelled curcumin to examine its tissue distribution. At dosages of 400, 80, or 10 mg of [3H] curcumin, they detected radioactivity in the blood, liver, and kidney. 12 days following the dose of 400 mg, significant levels of radiolabelled compounds were found in tissues. The proportion of curcumin absorbed (60–66 %) remained similar regardless of dosage, demonstrating that adding additional curcumin does not increase absorption (Ataie *et al.*, 2010). In other words, bioavailability in rats is a dose-dependent limitation. Low intrinsic activity, a high rate of metabolism, the inactivity of metabolic products, poor absorption, and/or quick excretion and clearance from the body are all factors for lower bioavailability within the body. However, studies on the absorption, distribution, metabolism, and excretion of curcumin over the last three decades have demonstrated poor absorption and rapid metabolism of curcumin, severely limits its bioavailability.

CONCLUSION

Curcumin greatly improves immunity, health, and flesh nutrient quality by simply introducing it into an aqueous medium of *H. fossilis*, according to bioscreening outcomes (Pandey *et al.*, 2022). As a result, aquaculture practices can be improved by reducing the human health risks associated with nutrient-depleted fish. Curcumin bioaccumulation may be concentration-dependent with duration in this study. However, its clearance from the system avoids any type of ailment in physiology due to over bioaccumulation; this makes this bio compound even more important and has aquaculture significance.

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Conflict of interest

The authors declare no conflict of interest

Data availability

Data supporting these findings are available within the article or upon request

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