THE AMELIORATIVE POTENTIAL OF *VERNONIA AMAGDALINA* ON THE AMINO ACID PROFILE OF *CLARIAS GARIEPINUS* (BURCHELL 1822) EXPOSED TO DICLOFENAC

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ABSTRACT

The inappropriate disposal of wastes causes an imbalance in natural aquatic ecosystems, affecting the marine environments, human health, and productive activities. This present study was designed to investigate the ameliorative effect of a medicinal plant, bitter leaf extract (BLE) on African catfish (Clarias gariepinus) fingerlings contaminated with diclofenac (DFC) using amino acid profile indices. The fingerlings were exposed to different concentrations of both BLE (6% body weight) and DCF (0.4mg/L) after a series of range-finding tests. Liver tissues of the experimental fishes were collected on the 14th and 28th day and were analyzed for the assessment of amino acid profile. The result obtained on the amino acid profile of C. gariepinus showed a significant increase ($p<0.05$) in the treatments with BLE (1.06 ± 0.04 to 15.22 ± 0.01) and DCF (0.48 ± 0.04 to 14.79 ± 0.04) when compared to their control group. Glycine, alanine, serine, glutamate, arginine, tyrosine, leucine and histidine showed a significant increase ($p<0.05$) while cystine and methionine showed a significant decrease ($p<0.05$) when compared to their control groups (0.29 ± 0.00 to 13.77 ± 0.04). Threonine, valine, isoleucine, tryptophan, phenylalanine, aspartate, proline and lysine varied when compared to their control groups. The results indicate that the given concentration of DCF and V. amygdalina may have no adverse effect on the amino acid profile of C. gariepinus. However, this study has shown the ameliorative potential of V. amygdalin against the effect of DCF in C. gariepinus.

Keywords: Aquatic ecosystem, Ameliorative effect, Catfish, Bitter leaf extract, Diclofenac, Amino acids

INTRODUCTION

In recent years, the health of the ecosystem has become a growing concern all over the world both locally and internationally. Water as a natural resource, is a part of the ecosystem, which is essential for the maintenance of life. However, water can be a means of transmitting diseases when contaminated (Mouly *et al*., 2018). According to Mateo-Sagasta *et al*. (2017), most aquatic ecosystems have a natural tendency to dilute contaminants to some extent, but severe toxins, interrupt the proper functioning of plants and animals and consequently, cause damage to the man exploiting the resources (Bashir *et al*, 2020). Toxic substances enter the aquatic ecosystem through agricultural, industrial, and domestic activities (Kumar *et al*., 2021); however, affect the general health of living organisms and render water bodies unsuitable for survival and consumption (Bashir *et al.,* 2020).

Agricultural and pharmaceutical products, which are called, emerging contaminants, are the

main sources of contaminants to the aquatic environment (Kraemer *et al*., 2019). The extensive use of pharmaceutical products in both human and veterinary medicine has led to contamination of aquatic ecosystems. Pharmaceutical drugs have become the focus of environmental concern as they are not completely metabolized or degraded, thereby producing chemical residues, which persist in the environment for a long time and cause harm to the environment (Carey and McNamara, 2015; Boyd and McNevin, 2015); mainly in open water where the chemicals are not controlled easily (Noga, 2010). They affect the non-target organisms directly or indirectly (Panwar *et al*., 2020). However, one of the common pharmaceutical drugs that contaminate the aquatic environment is diclofenac (DFC). The name "diclofenac" was derived from its chemical name: 2-(2, 6-dichloranilino) phenylacetic acid. DFC was discovered by Ciba-Geigy, a Swiss pharmaceutical company in 1973 (now merged with Novartis) (Lonappan *et al*., 2016). It was first approved by the FDA in July 1988 under the trade name Voltaren, marketed by Novartis (previously Ciba-Geigy) (Brogden *et al*., 1980). DFC is a therapeutic anti-inflammatory and analgesic drug in the world and one of the most common pharmaceutical drugs currently detected in the environment (UNESCO and HELCOM, 2017). It is one of the most used and widely sold anti-inflammatories and analgesics in the world and has been utilized for an extended period. It has been widely detected in aquatic environments and at concentrations that can be indicative of detrimental environmental effects (Fick *et al*., 2009). The presence of contaminants in aquatic environments, even at low concentrations, causes negative impacts on aquatic organisms (Grenni *et al*., 2018; Kumar *et al*., 2019).

Diclofenac is a phenylacetic acid derivative and a non-steroidal anti-inflammatory drug (NSAID) that is used for the treatment of rheumatic diseases, minor and medium pain, and post-surgery analgesia in medicine (Kasperek *et al*., 2015) and to reduce inflammation or as a pain reliever in diseased conditions, such as arthritis or acute injury (Hunter *et al*., 2011). It is used commonly to treat mild to moderate post-operative or post-traumatic pain, particularly when inflammation is also present (Ofokansi and Kenechukwu, 2011). Reports have shown that it is used in rheumatoid arthritis, degenerative joint disease, ankylosing spondylitis, osteoarthritis, and allied conditions, and in the treatment of pain resulting from minor surgery, trauma, dysmenorrhoea, migraines, and menstrual pain (Brogden *et al*., 1980; Ofokansi and Kenechukwu, 2011). It is also used as anti-uricosuric and analgesic (Lonappan *et al*., 2016). It is often used in combination with misoprostol to prevent NSAID-induced gastric ulcers (Brogden *et al*., 1980). DFC can be applied to the skin or it can be administered orally. DFC is the most potent NSAID on a molar basis and is among the better-tolerated NSAIDs (Tuncay *et al*., 2000a, b; Kumbar *et al*., 2002; Ofokansi and Kenechukwu, 2011). It is the most commonly used NSAID and is often seen as the 'world's most popular pain killer' with a market share close to that of the next three most popular drugs combined (ibuprofen, mefenamic acid, and naproxen) (McGettigan and Henry, 2013; Lonappan *et al*., 2016).

Clarias gariepinus Burchell 1822 (Siluriformes: Clariidae), commonly known as African catfish, is an African fish species that is popular among fish farmers and consumers (Okukpe *et al*., 2018). The name is derived from the Greek *chlaros*, which means lively, about the ability of the fish to live for a long time out of water (Froese and Pauly, 2011). *C. gariepinus* is known for its cylindrical body with scaleless skin and elongated spineless dorsal fin (USFWS, 2022). *C. gariepinus* is used in this study because of its hardiness and easy acclimation to laboratory conditions. This study aims to examine the ameliorative effects of BLE on the amino acid profile of *C. gariepinus* exposed to varied concentrations of DCF.

MATERIALS AND METHODS

Experimental Plant Material and Preparation of Leaf Extract: The experiment was carried out at the Applied Biology Special Laboratory, Enugu State University of Science and Technology (ESUT), Agbani, Enugu, Enugu State. Fresh bitter leaves were obtained from a local farm and were identified (Dalziel, 1937) and

authenticated by a taxonomist in the Applied Biology and Biotechnology Department, Enugu State University of Science and Technology, Enugu. The voucher specimen (ESUT Herbarium CAB 2/Number 53) was kept in the Departmental herbarium for referral purposes. The leaves were washed with distilled water and air dried for two weeks under shade, then ground into fine powder using a sterile pestle and mortar, and stored in an air-tight container for further use. The ethanolic bitter leaf extract (BLE) was prepared according to Abdel-Shafi *et al*. (2019) and Omar *et al*. (2020). Bitter leaf powder (100 g) was extracted with 70% v/v aqueous ethanol for two hours. One hundred grams of bitter leaf powder yielded 20 g ethanolic extract.

Experimental Chemical: For the present study, Diclofenac Potassium Tablets USP 50 mg with the brand name Chloflam 50, manufactured by McCoy Pharma Private Limited, Palghar, Maharashtra-401506, India with the Batch No: MP9574, Serial No: OMCPL5AA6040 and NAFDAC Reg No: 04-5388, was used for the study.

Experimental Fish: One hundred (150) 8-week-old healthy *C. gariepinus* fingerlings with a mean weight of 1.31 ± 0.14 g were purchased from Sacen Fish Farm, Enugu, Nigeria and transported in a well-aerated 50-litre capacity aquarium tank to the Applied Biology Special Laboratory, Enugu State University of Science and Technology, Agbani, Enugu State, Nigeria. The fish were acclimatized to laboratory conditions for two weeks using well water and were fed 3% of their body weight in divided rations, twice daily (8:30 am and 5:00 pm) with Skretting Catfish Starter Feed (45% crude protein and 2000 kcal/kg metabolisable energy), Skretting Nigeria, Ibadan, Nigeria.

Experimental Diets and Design: The feed was mixed with either distilled water or BLE according to Dandi *et al*. (2022) with little modification. One kilogram of the commercial feed was weighed into each of two different basins. One of the basins held the control (CT) feed, to which 100 mL of distilled water was added and mixed thoroughly; the feeds were pelletized and dried under shade, and later stored. The experimental diets were made indoors by adding to the commercial feed 6% (60 mL; high dose) BLE. The experimental diets were similarly pelletized and dried under shade, and stored in plastic bags in a refrigerator for later use.

After acclimatization, fish were randomly divided into four groups; A, B, C, and D, and replicated thrice (10 fishes/replicate). The treatments were; A (control) (0 ml), B (6% BLE + 0.4 DCF), C (6% BLE), and D (0.4 DCF). Group A (control, 0 ml) and D (0.4 DCF) were fed with the control diet (CT) without and with exposure to 0.4 mg/L of DFC, respectively. Groups B and C were fed with 6% BLE-supplemented diets without and with exposure to 0.4 mg/L of DFC, respectively. Test fish were monitored at regular time intervals during the experimental period (28 days) and liver samples were taken on the 14^{th} and 28^{th} days for amino acid profile analysis.

Determination of Amino Acids: Amino acid analysis was done by ion exchange HPLC chromatography (Benitez, 1989), using the Applied PTH Amino Acid Analyzer (Model 120A). About 2 g of each of the samples was defatted using chloroform/methanol (2:1) (Horwitz and Latimer, 2006) and then hydrolyzed at 110^{o} C under a nitrogen atmosphere for 22 hours with 6 M hydrochloric acid. seventeen amino acids (glycine, alanine, serine, proline, aspartate, glutamate, arginine, tyrosine, cystine, isoleucine, leucine, threonine, valine, histidine, phenylalanine, methionine and lysine) were identified. For tryptophan determination, 2 g of each of the samples was separately hydrolyzed with 4.2 M sodium hydroxide for 22 hours and were then neutralized to pH 7.0 with 6 M of hydrochloric acid. These hydrolysates were then injected into the amino acid analyzer for separation and characterization (Oriolowo *et al*., 2020). Quantification was obtained by using external amino acid standards and the results were corrected for the recoveries. All analyses were conducted in triplicate for each sample.

Data Analysis: Biological data from the feeding trial were subjected to a one-way analysis of variance (ANOVA) using the statistical package for Social Sciences (SPSS) Version 21. Where

significant differences were observed, the means were compared further using the Duncan New Multiple test range at a 5% level of significance.

RESULTS

The effects of *V. amagdalina* extract on the amino acid profile of *C. gariepinus* are presented in Table 1. On day 14 for 6% BLE + 0.4 DCF, when compared to the control, there was a significant increase in all the amino acids except aspartate, cystine, tryptophan, phenylalanine and methionine that had a significant decrease ($p<0.05$) values. In contrast, tyrosine had no significant difference ($p>0.05$). For 6% BLE, when compared to the control, there was a significant increase ($p<0.05$) in all the amino acids except glycine, aspartate, cysteine, leucine, tryptophan, methionine and lysine which significantly decreased ($p<0.05$). For the 0.4 DCF, there was a significant increase ($p<0.05$) in glycine, serine, glutamate, arginine, tyrosine, leucine threonine and histidine and a significant decrease ($p<0.05$) in proline, aspartate, cysteine, isoleucine, valine, tryptophan, phenylalanine and lysine.

On day 28 for 6% BLE + 0.4 DCF, when compared to the control, there was a significant increase ($p<0.05$) in glycine, alanine, serine, glutamate, arginine tryptophan histidine and phenylalanine. Equally, a significant decrease ($p<0.05$) in proline, tyrosine, cystine isoleucine, threonine and valine levels were recorded. For 6% BLE, there was a significant increase ($p<0.05$) in all the amino acids except cystine, isoleucine, methionine and lysine had a significant decrease ($p<0.05$) and tryptophan with no significant difference ($p>0.05$). For 0.4 DCF, there was a significant increase ($p<0.05$) in all the amino acid levels except, isoleucine and methionine which had a significant decrease ($p<0.05$) while cystine had no significant difference ($p>0.05$).

Alanine, on day 14, had its lowest value in control and 0.4 DCF (1.87 ± 0.04 mg/L) and its highest value in 6% BLE + 0.4 DCF (6.319 ± 0.04 mg/L). On day 28, its lowest value was in control (2.21 ± 0.00 mg/L) and its highest value was in 6% BLE (4.22 ± 0.00 mg/L).

Serine, on day 14, had its lowest value in control (1.56 ± 0.02 mg/L) and its highest value in 6% BLE + 0.4 DCF (6.32 ± 0.04 mg/L). On day 28, its lowest value was in control (2.21 ± 0.00 mg/L) and its highest value was in 6% BLE (4.22 ± 0.00 mg/L).

Proline, on day 14, had its lowest value in 0.4 DCF (1.81 ± 0.03 mg/L) and its highest value in 6% BLE + 0.4 DCF (4.59 ± 0.03 mg/L). While in day 28, its lowest value was at 6% BLE + 0.4 DCF (1.34 ± 0.00 mg/L), and its highest value was at 0.4 DCF (4.30 ± 0.04 mg/L).

Valine, on day 14, had its lowest value in 0.4 DCF (1.20 ± 0.03 mg/L) and its highest value in 6% BLE + 0.4 DCF (4.21 ± 0.03 mg/L). on day 28, its lowest value was at 6% BLE + 0.4 DCF (0.74 ± 0.00 mg/L), and its highest value was at 6% BLE (4.11 ± 0.03 mg/L).

Thereonine, on day 14, had its lowest value in control (3.59 ± 0.03 mg/L) and its highest value in 0.4 DCF (4.60 ± 0.03 mg/L). On day 28, its lowest value was at 6% BLE + 0.4 DCF (3.19 ± 0.00 mg/L) and its highest value was at 6% BLE (4.37 ± 0.00 mg/L).

Isoleucine, on day 14, had its lowest value in 0.4 DCF (1.51 ± 0.02 mg/L) and its highest value in 6% BLE + 0.4 DCF (4.30 ± 0.02 mg/L). on day 28, its lowest value was in 6% BLE + 0.4 DCF (4.13 ± 0.00 mg/L) and its highest value was in control (4.89 ± 0.00 mg/L).

Leucine, on day 14, had its lowest value in 6% BLE (7.03 ± 0.03 mg/L) and its highest value in 6% BLE + 0.4 DCF (8.21 ± 0.03 mg/L). On day 28, its lowest value was in control (1.43 ± 0.00 mg/L) and its highest value was in 6% BLE (7.78 ± 0.00 mg/L).

Aspartate, on day 14, had its lowest value in 6% BLE+ 0.4 DCF (0.67 ± 0.04 mg/L) and its highest value in control (1.92 ± 0.04 mg/L). On day 28, its lowest value was in control (1.68 ± 0.06 mg/L) and its highest value was in 6% BLE (12.10 ± 0.00 mg/L).

Lysine, on day 14, had its lowest value in 0.4 DCF (4.83 ± 0.03 mg/L) and its highest value in 6% BLE +0.4 DCF (9.37 ± 0.03 mg/L). On day 28, its lowest value was at 6% BLE + 0.4 DCF (2.42 ± 3.42 mg/L) and its highest value was at 6% BLE (6.31 ± 0.00 mg/L).

Table 1: The effect of bitter leaf extract on the amino acid profile of *Clarias gariepinus* exposed to diclofenac

Parameters	Exposure time	Concentration (mg/l)			
		Control	6% BLE + 0.4 DCF	6% BLE	0.4 DCF
Glycine	14 days	1.24 ± 0.04^{a}	4.24 ± 0.04^{d}	3.92 ± 0.04^{c}	1.51 ± 0.04^{b}
	28 days	1.38 ± 0.00 ^{a}	1.55 ± 0.00^{b}	4.05 ± 0.00^{d}	3.29 ± 0.00^{c}
Alanine	14 days	1.87 ± 0.04^{a}	6.32 ± 0.04^{c}	3.51 ± 0.04^{b}	1.87 ± 0.04^{a}
	28 days	2.21 ± 0.00^{a}	2.96 ± 0.00^{b}	4.22 ± 0.00^{d}	3.56 ± 0.00^{c}
Serine	14 days	1.56 ± 0.02^{a}	4.17 ± 0.03^{d}	4.05 ± 0.03^{c}	1.73 ± 0.03^{b}
	28 days	1.39 ± 0.00^{a}	1.65 ± 0.00^{b}	5.47 ± 0.00^{d}	4.47 ± 0.00^{c}
Proline	14 days	3.10 ± 0.03^{b}	4.59 ± 0.03^{d}	4.20 ± 0.03^{c}	1.81 ± 0.03^{a}
	28 days	1.68 ± 0.00^{b}	1.33 ± 0.00^{a}	4.03 ± 0.00^{c}	4.30 ± 0.04^{d}
Aspartate	14 days	1.92 ± 0.04^{d}	0.67 ± 0.04^{a}	1.17 ± 0.04^{b}	1.52 ± 0.04^{c}
	28 days	1.68 ± 0.06^{a}	1.69 ± 0.01^{a}	12.10 ± 0.00^{c}	11.17 ± 0.04^{b}
Glutamate	14 days	13.77 ± 0.04^{a}	14.49 ± 0.04^{c}	14.03 ± 0.04 ^{b}	14.79 ± 0.04^{d}
	28 days	4.14 ± 0.00^{a}	16.84 ± 0.00^{d}	15.22 ± 0.01^{c}	14.42 ± 0.04^{b}
Arginine[1]	14 days	2.75 ± 0.02^{a}	6.57 ± 0.02^{d}	4.99 ± 0.02^{c}	2.91 ± 0.02^{b}
	28 days	1.45 ± 0.00^{a}	2.78 ± 0.00^{b}	6.24 ± 0.00^{d}	5.00 ± 0.04^{c}
Tyrosine	14 days	2.93 ± 0.01^{a}	2.93 ± 0.01^{a}	3.31 ± 0.01^{b}	3.40 ± 0.01^{c}
	28 days	1.54 ± 0.00^{b}	0.21 ± 0.25^{a}	2.98 ± 0.00^{c}	3.32 ± 0.04^{d}
Cystine	14 days	3.71 ± 0.04^{c}	1.40 ± 0.04^{b}	1.36 ± 0.04^{b}	1.04 ± 0.04^{a}
	28 days	1.31 ± 0.01^{c}	0.03 ± 0.03^{a}	1.23 ± 0.00^{b}	1.36 ± 0.04^{c}
Isoleucine[1]	14 days	1.86 ± 0.02^{b}	4.30 ± 0.02^{d}	4.12 ± 0.02^{c}	1.51 ± 0.02^{a}
	28 days	4.89 ± 0.00^{d}	4.13 ± 0.00^{a}	4.83 ± 0.00^{c}	4.20 ± 0.04^{b}
Leucine[1]	14 days	7.67 ± 0.03^{b}	8.21 ± 0.03^{d}	7.03 ± 0.03^{a}	7.86 ± 0.03^{c}
	28 days	1.43 ± 0.00^{a}	1.49 ± 0.00^{a}	7.78 ± 0.00^{b}	7.42 ± 0.35^{b}
Threonine[1]	14 days	3.59 ± 0.03^{a}	4.41 ± 0.03^{c}	4.21 ± 0.03^{b}	4.60 ± 0.03^{d}
	28 days	3.84 ± 0.00^{b}	3.19 ± 0.00^{a}	4.37 ± 0.00^{c}	4.31 ± 0.04^{c}
Valine[1]	14 days	1.77 ± 0.03^{b}	4.21 ± 0.03^{d}	4.11 ± 0.03^{c}	1.20 ±0 .03^{a}
	28 days	0.89 ± 0.00^{b}	0.74 ± 0.00^{a}	5.26 ± 0.00^{d}	4.60 ± 0.04^{c}
Tryptophan[1]	14 days	1.81 ± 0.04^{c}	1.11 ± 0.04 ^{b}	1.06 ± 0.04^{b}	0.48 ± 0.04 ^{a}
	28 days	1.15 ± 0.00^{a}	2.45 ± 0.00^{c}	1.18 ± 0.00^{ab}	1.23 ± 0.04^{b}
Histidine[1]	14 days	0.78 ± 0.04^{a}	3.12 ± 0.04^{d}	2.30 ± 0.04^{c}	1.01 ± 0.04^{b}
	28 days	0.29 ± 0.00^{a}	0.99 ± 0.00^{b}	2.95 ± 0.00^{c}	2.91 ± 0.04^{c}
Phenlalanine[1]	14 days	3.75 ± 0.01^{c}	3.10 ± 0.01^{a}	5.17 ± 0.01^{d}	3.61 ± 0.01^{b}
	28 days	0.48 ± 0.05^{a}	0.74 ± 0.00^{b}	5.34 ± 0.00^{d}	5.18 ± 0.04^{c}
Methionine[1]	14 days	1.52 ± 0.04^{b}	1.11 ± 0.04^{a}	1.12 ± 0.04^{a}	1.59 ± 0.04^{b}
	28 days	4.41 ± 0.03^{c}	4.43 ± 0.00^{c}	1.60 ± 0.02^{b}	1.40 ± 0.04^{a}
Lysine[1]	14 days	6.83 ± 0.03^{c}	9.37 ± 0.03^{d}	6.30 ± 0.03^{b}	4.83 ± 0.03^{a}
	28 days	4.46 ± 0.00^{ab}	2.42 ± 3.42^{a}	6.31 ± 0.00^{b}	6.29 ± 0.04^{b}

Means with different letters superscripts within each row are significantly different (p≤0.05). Data are presented as mean ± SE, [1]Essential amino acid

Methionine, on day 14, had its lowest value in 6% BLE + 0.4 DCF (1.11 ± 0.04 mg/L) and its highest value in 0.4 DCF (1.59 ± 0.04 mg/L). On day 28, its lowest value was 0.4 DCF (1.40 ± 0.04 mg/L) and its highest value was in 6% BLE + 0.4 DCF (4.43 ± 0.00 mg/L).

Glutamate, on day 14, has its lowest value in control (13.77 ± 0.04 mg/L) and its highest value in 0.4 DCF (14.79 ± 0.04 mg/L). On day 28, its lowest value was in control (4.14 ± 0.00 mg/L) and its highest value was in 6% BLE + 0.4 DCF (16.84 ± 0.00 mg/L).

Phenylalanine, on day 14, had its lowest value in 6% BLE + 0.4 DCF (3.10 ± 0.01 mg/L) and its highest value in 6% BLE (5.17 ± 0.01 mg/L). On day 28, its lowest value was in control (0.48 ± 0.05 mg/L) and its highest value was in 6% BLE (5.34 ± 0.00 mg/L).

Histidine, on day 14, had its lowest value in control (0.7840 ± 0.04 mg/L) and its highest value in 6% BLE + 0.4 DCF (3.12 ± 0.04 mg/L). On day 28, its lowest value was in control (0.29 ± 0.00 mg/L) and its highest value was in 6% BLE (2.95 ± 0.00 mg/L).

Arginine, on day 14, had its lowest value in control (2.75 ± 0.02 mg/L) and its highest value in 6% BLE + 0.4 DCF (6.57 ± 0.02 mg/L). On day 28, its lowest value was in control (1.45

± 0.00 mg/L) and its highest value was in 6% BLE (6.24 ± 0.00 mg/L). On day 14, tyrosine had its lowest value in control and 6% BLE + 0.4 DCF (2.93 ± 0.01 mg/L) and its highest value in 0.4 DCF (3.40 ± 0.01 mg/L). On day 28, its lowest value was at 6% BLE + 0.4 DCF (0.21 ± 0.25 mg/L) and its highest value was at 0.4 DCF (3.32 ± 0.04 mg/L).

Tryptophan, on day 14, had its lowest value in 0.4 DCF (0.48 ± 0.04 mg/L) and its highest value in control (31.81 ± 0.04 mg/L). On day 28, its lowest value was in control (1.15 ± 0.00 mg/L) and its highest value was in 6% BLE + 0.4 DCF (2.45 ± 0.00 mg/L).

Cystine, on day 14, had its lowest value in 0.4 DCF (1.04 ± 0.04 mg/L) and its highest value in control (3.71 ± 0.04 mg/L). On day 28, its lowest value was at 6% BLE + 0.4 DCF (0.03 ± 0.03 mg/L) and its highest value was at 0.4 DCF (1.36 ± 0.04 mg/L).

DISCUSSION

Eighteen (18) amino acids (essential and non-essential) were identified in *C. gariepinus* at different proportions. Amino acid is an important component of the healing process; hence, a deficiency of amino acids will hinder the recovery process (Tenyang *et al*., 2014). From the result, glutamate had the highest value. This is in line with the reports by Tenyang *et al*. (2014), Abdulkarim *et al*. (2017) and Adepoju *et al.* (2022). This supports the finding that glutamate is the most concentrated amino acid composition in fish (Adepoju *et al*., 2022). The result showed a significant increase of amino acid in fish treated with BLE and DCF when compared to the control. This is similar to the report of Sowunmi *et al*. (2020) and Okpe *et al*. (2022), who recorded a significant increase in protein levels in mice and broiler chickens. This is also in line with Palaniappan and Muthulingam (2016) who observed an increase in amino acid contents in the gill, liver and kidney of *C. striatus*. The increase in amino acid may mean that the cell can quench the effect of free radical and peroxide processes, which could ultimately result in modulating the host antioxidant status (Sowunmi *et al*., 2020).

According to Usydus *et al*. (2011), the nutritional properties of fish make them valuable and beneficial for human health. From the result of this study, it has been observed that glutamate had the highest value (4.14 ± 0.00 to 16.84 ± 0.00 mg/g). This was in line with the reports of Aremu *et al*. (2013), Tenyang *et al*. (2014), Abdulkarim *et al*. (2017) and Adepoju *et al.* (2022). This shows that glutamate is the most concentrated amino acid composition in fish (Aremu *et al*., 2013; Adepoju *et al*., 2022). Glutamate is essential for proper brain functioning as it is used to form memories (Adepoju *et al*., 2022). Thus, a low level of glutamate can cause the problem in the brain. Reports have shown that glutamate is the major excitatory neurotransmitter in the mammalian central nervous system. Hence, an increase in the level of glutamate improves brain functions (Zhou and Danbolt, 2014; Adepoju *et al*., 2022). Abdulkarim *et al*. (2017) reported that glutamic acid is an important source of nitrogen, and is usually used to improve the taste of food in the form of monosodium glutamate a "Mchuzi mix" commonly used in Asia and West Africa. Lysine is an important precursor for the de novo synthesis of glutamate (Abdulkarim *et al*., 2017). A reduced supply of lysine in the diet may lead to mental and physical retardation. According to Chapman *et al*. (2010), lysine is an amino acid that is limited in a cereal-based diet consumed by many human populations. Deficiency of lysine in plant proteins such as cereals can thus be supplemented by consuming fish.

Amino acids are the important components of healing processes and any deficiency in these essential components will hinder the recovery process (Tenyang *et al*., 2014). In addition to glycine, which is one of the major components of human skin collagen, together with other amino acids such as alanine, proline, arginine, serine, isoleucine, and phenylalanine form a polypeptide that will promote regrowth and tissue healing (Witte *et al*., 2002). The fish treated with BLE and DFC separately showed a significant increase in these amino acids on day 28 except isoleucine. The fishes treated with both BLE and DFC together show a significant increase in these amino acids on day 28 except proline and isoleucine.

Conclusion: The result from this study showed that the given concentration of diclofenac had no adverse effect on the amino acid profile of *C. gariepinus,* but caution should be exercised in the clinical use of DFC for therapeutic purposes. The use should be limited to the lowest dose and treatment duration required to achieve the best therapeutic effect. The result from this study indicated that dietary supplementation with BLE at the given concentration tends to improve the amino acid profile of *C. gariepinus*. This current study has shown the ameliorative potential of BLE against the effect of diclofenac on the amino acid profile of *C. gariepinus*. However, more research is necessary to investigate the possible effect of BLE (in varying dosages) on fishes treated with diclofenac on their amino acid profile and other parameters, such as haematology, histopathology, biochemistry, oxidative stress, metabolism and digestion.

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