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Antioxidant and protective properties of fish meal containing different inclusion levels of *Sesame indicum* (Beni seed) on acetaminophen-induced oxidative stress in *Clarias gariepinus*

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Abstract
This study analyzed the antioxidant and protective properties of fish meal containing different inclusion levels of *Sesame indicum* (Beni seed) on acetaminophen-induced oxidative stress in *Clarias gariepinus*. A total of 140 of *C. gariepinus* with 332.16.5±5.20g and 34.25±0.43cm as mean weight and length were grouped into 7 tanks with 20 *C. gariepinus* per tank. Each tank was fed separate formulated diet, namely; DT1 (commercial diet plus acetaminophen), stand as the negative control group, DT2 (commercial diet only), as a positive control, DT3 (0% *S. indicum* with 100% soya bean meal, DT4 (25% *S. indicum* with 75% soya bean meal, DT5 (50% *S. indicum* with 50% soya bean, DT6 (75% *S. indicum* with 25% soya bean and DT7 (100% *S. indicum* with 0% soya bean for four (4) weeks. Antioxidant properties of the prepared diets, the serum marker enzymes, and chemistry of *C. gariepinus* fed different diets were examined. High levels of serum marker enzymes, blood glucose, serum bilirubin, total cholesterol, and creatinine and a significant decreased in serum total protein and albumin were observed in *C. gariepinus* fed control diet plus acetaminophen compared to those fed the control diet (DT2) only. Inversely, low levels of serum marker enzymes, blood glucose, serum bilirubin, total cholesterol, and creatinine, and increase levels of total protein and albumin were detected in *C. gariepinus* fed formulated diets with varying grades of *S. indicum* inclusion plus acetaminophen compared to those fed commercial diet plus acetaminophen intubation. The findings of this work showed that all formulated diets with varying percentages of *S. indicum* inclusion are good macrocellular damage repairer in stressful *C. gariepinus*. However, formulated diets (DT5 and DT6) looked more promising in combating oxidative stress related disorders in *C. gariepinus*.

Keywords: Acetaminophen, antioxidant property, *C. gariepinus*, fish meal, oxidative stress, *S. indicum*

1. Introduction

Involvements of oxidative stress in more than one hundred (100) diseases have been documented [1]. Many types of ROS-scavengers (antioxidants) reported being acquired from Natural foodstuffs, fruits and vegetables [2]. Reduction/prevention of reactive oxygen species (ROS) productions in animals by consuming plant produces that contain high ROS-neutralizers have been reported [3]. High intakes of dietary antioxidants are recommended where the defense mechanisms of natural antioxidant are not sufficient [4]. The conventional damages to the liver do create a response termed inflammation. It simply means to expose the causative agent and eliminate it if possible to heal the damage [5]. Prostaglandin is a vital mediator for inflammation and its prevention is a way forward for treatment [6]. In acute and chronic inflammation, the drugs recommended for use include; aspirin, ibuprofen, paracetamol, etc. Paracetamol scientifically called acetaminophen is generally used against pains [7]. Its mechanisms of action are still unclear. Although its mechanism of action of preventing prostaglandin production is it accepted primary site of action [8]. The drug dose and the patient's age are two (2) factors that determined the metabolism of paracetamol [9]. Paracetamol metabolizes into cysteine metabolites and glucoronide sulphate in a healthy individual. It is relatively safe when the therapeutic doses are taken but in high doses, it becomes hepatotoxic [10]. The non-toxic metabolites generated during paracetamol metabolism was majorly by the conjugation process in the liver [7]. There are used to be conjugation saturation that produces excess acetyl para amino phenol (APAP) during acute mega doses of the drug. The APAP produced is converted into a metabolite that is toxic to damage the liver through the activity of enzymes of cytochrome [11]. These conventional drugs used in combating oxidative stress-related diseases are good in generating ROS, which are the source of oxidative stress-related diseases in living tissues [4].

Sesamum indicum is cheap, naturally blessed foodstuff with abundant nutrients provided for the inhabitants [12]. It is a member of the *Pedaliaceae* family, which is cultivated much in Asian and African countries [13]. *S. indicum* has good eatable oil, protein, mineral (calcium) and antioxidants and has been recommended as good food for ages [14]. The fried and fermented seeds are used in making soup for human consumption [15]. Its consumption in Nigeria is highly demanding due to global decrease in animal proteins, high demand for cholesterol-free and saturated fats lower foods. The sesame food acceptability in Nigeria may reflect its health benefit recognition [12]. Its nutraceutical uses include cancer prevention (myristic acid) and heart disease. Sesame seed consumption appears to increase plasma gamma-tocopherol and enhanced a vitamin E activity which is believed to prevent cancer and heart disease [16]. Beni seed has been loudly used as a protein supplement in the diet of fishes. This is because of the nutritional content that is favorable for the digestibility, growth, and feed utilization among domesticated animals [16].

Fisheries have played a very vital role in improving the food security status of the people; it contributes about 15-16% to the total animal protein consumed by 2.9 billion people in low-income and food-deficient countries [17]. Fishmeal is the preferred protein ingredient in aquaculture feed and contributes significantly to the variable production cost in the aquaculture industry. However, decreasing fishmeal supply relative to demand and increasing costs threaten the sustainability and growth of the aquaculture industry [17]. Lack of good quality feed for economic production adversely increases diseases manifestation and reduce the total harvest of fish [18]. The success of fish farming invariably depends on the provision of suitable and economical fish feed with animal or plant-based protein and antioxidants sources [19]. The development of a more suitable aquaculture feed production will depend on identifying and establishing alternative feedstuffs to fishmeal [20]. This research considered the inclusion of *S .indicum* (Beni seed) powder as a source of proteins and antioxidants. Past studies have demonstrated that complete or partial substitution of fishmeal with alternative proteins and antioxidants does not adversely affect fish wellness and production [17]. Hence, promoting the inclusion of locally available antioxidant and protein-rich stuff in fish meals may increase the fish health and production performance. Therefore, antioxidant levels of fishmeal will eliminate stress and disease conditions that might affect their production performance. This study was therefore carried out to evaluate the antioxidant and protective ability of fish meals with varying percentages of *S. indicum* inclusion in *C. gariepinus*.

2. Materials and Methods

2.1 Sample Preparation

The feedstuffs were finely grounded and mixed in the plastic bowl into dough form using hot water, with cassava starch as binding material. The mixture was then pelleted by passing it through a mincer of 2 mm die to produce 2 mm diameter size of pelleting machine (Hobart A-200T GmbH, Rhen-Bosch, Offenburg, Germany). These were sundried to about 10% moisture content, packed in polythene bags and kept safe dry for use. The soya bean meal consisted of fish meal, soya bean, maize and other ingredients as presented in table 1.

Table 1: Formulation of soya beans meal

Ingredients	Combination (%)
Soya beans	48.4
Fish meal	21.5
Maize	11.4
Groundnut cake	15.2
Vitamin premix	1.3
Sodium chloride	2.2
Total	100

2.1.1 Feed Formulation

Briefly, about five (5) experimental diets at varying sesame seed and soya beans meal compositions, namely; diet 1, 2, 3, 4, 5, and 6 were formulated (Table 2). The diet 1 is commercial diet (control), Diet 2 is composed of 0% sesame seed meal with 100% soya bean meal, Diet 3 is having 25% sesame seed meal with 75% soya bean meal, Diet 4 is composed of 50% sesame seed meal with 50% soya bean meal, Diet 5 is composed of 75% sesame seed meal with 25% soya bean meal, Diet 6 is 100% sesame seed meal with 0% soya bean meal.

Table 2: Experimental diet formulation

Diet	Formulated diet (%)					
	FD1	FD2	FD3	FD4	FD5	FD6
Sesame seed	CD	0	25	50	75	100
Soya bean meal		100	75	50	25	0

CD: commercial diet

2.2 Animals

A total of one hundred and forty (140) of Catfish (*C. gariepinus*) of mixed-sex and the same age (mean weight of 332.16.5±5.20g and length of 34.25±0.43) were purchased from Lapai Gwari fish Village, along Lapai – Paiko-Minna road, Niger state. The fishes were transported in an aerated aquarium into the department of Biology, Ibrahim Badamasi Babangida University, Lapai, Niger State. Fishes were allowed to acclimatize to experimental conditions for one week before the feeding trial and were fed commercial fish feed meal (2mmVital feed, company, and country) three times daily. After the acclimatization, the fishes were starved for 24 hours. The water temperature, pH level, hardness of water and available oxygen of the aquarium were monitored throughout the experimental period. The leftover feed in the aquarium water was siphoned every day to avoid infections and mortality during the period of the experiment. The paracetamol-induced oxidative stress model was achieved using the method of Ndatsu *et al*. [4]

2.2.1 Experimental Treatment and Design

The fishes were selected randomly into seven (7) rearing tanks with 20 *C. gariepinus* per rearing tank [21]. Each rearing tank (RT1, RT2, RT3, RT4, RT5, RT6, and RT7) was assigned to a dietary treatment as shown in Table 2, and all were replicated three (3) times and covered with a net to prevent the predators. The feeding trial commences after fish were starved for 24 hours as follows:

Table 3: Feeding trial and treatment

RT	DT (20g/day)	NF	AC (100 mg/g/day)
1	Commercial diet	20	+
2	Commercial diet	20	-
3	0% sesame and 100% soya bean	20	+
4	25% sesame and 75% soya bean	20	+
5	50% sesame and 50% soya bean	20	+
6	75% sesame and 25% soya bean	20	+
7	100% sesame and 0% soya bean	20	+

+: added, -: not added, RT: rearing tank, DT: dietary treatment, NF: number of fish, AC: acetaminophen

The fishes were fed an experimental diet (20 g) once (1) in a day for four (4) weeks. Animal experiments were conducted following the internationally accepted principle for laboratory animal use and care [22]. At the end of 4 weeks, the experimental fishes starved for 12 hr. before harvested for blood sampling [23]. The blood samples were taken to Clinical Pathology Laboratory and centrifuged to have serum for the analyses of Liver enzymes.

2.3 Method of analysis procedures and data analysis

The free soluble and bound polyphenols extractions, assay of total phenol contents, assay of reducing property and free radical scavenging capacity were conducted using a method modified by Ndatsu *et al.* [4]. Serum enzyme markers (aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), and bilirubin) concentrations were analyzed using Roche Modular Auto-analyzer (Cobas® 8000 modular analyzer series). Total protein, albumin, glucose, and total cholesterol were analyzed using standard random laboratory kits (Sigma-Aldrich, USA). The data recorded were presented as the mean±standard deviation and analyzed using a one-way analysis of variance (ANOVA). The least significant difference (LSD) test was used to evaluate the significant difference between control and experimental group. Differences were considered statistically significant at $P<0.05$.

3. Results

Table 4: Antioxidant Properties of Formulated Diet Studied

DT (%)	Dietary treatments				
	TP (%)	FSF (%)	FSB (%)	RFP (%)	RBP (%)
DT1	12.6±2.00^{c}	47.2±0.01^{d}	41.3±0.01^{d}	0.3±1.01^{d}	0.5±1.00^{d}
DT2	15.0±3.00^{b}	65.5±0.01^{c}	63.7±1.00^{c}	1.4±0.31^{c}	1.5±0.01^{c}
DT3	16.7±1.02^{a}	75.4±2.21^{b}	63.4±1.00^{c}	1.6±0.31^{b}	1.8±0.01^{b}
DT4	16.4±2.01^{a}	82.3±4.12^{a}	76.6±0.02^{b}	1.6±0.22^{b}	2.3±0.01^{a}
DT5	16.8±3.01^{a}	82.6±3.01^{a}	81.2±2.02^{a}	1.8±0.31^{a}	2.4±1.00^{a}
DT6	16.5±2.11^{a}	75.5±2.51^{b}	81.1±3.01^{a}	1.8±0.20^{a}	1.7±0.23^{b}

Data are presented as mean±standard deviation. Means with the different superscript letters within a column are significantly different ($p<0.05$). DT: dietary treatment, TP: total phenol, FSF: free radical scavenging ability of free soluble phenols, FSB: free radical scavenging ability of bound phenols, RFP: reducing power of free soluble phenols, RBP: reducing power of bound phenols, DT1: commercial diet only (control), DT2: 0% sesame seed meal, 100% soya bean meal and paracetamol, DT3: 25% sesame seed meal, 75% soya bean meal and paracetamol, DT4: 50% sesame seed meal, 50% soya bean meal and paracetamol, DT5: 75% sesame seed meal, 25% soya bean meal and paracetamol, DT6: 100% sesame seed meal, 0% soya bean meal and paracetamol.

Table 3 presented the result of the antioxidant properties of the fish diets substituted with the graded level of *S. indicum* inclusion. It reveals that all the formulated diets have their antioxidant properties increased significantly ($p<0.05$) compared to those of control diets (DT1). The total phenol contents recorded in DT3 (16.7 %), DT4 (16.4%), DT5 (16.8%) and DT6 (16.5%) did not differed significantly from each other, but differed from that of DT2 (15.0%). Thus, the diet (DT5) recorded a high numerical value of total phenol content than others (Table 4). High free radical for soluble polyphenol (FSF) and the reducing properties for bound polyphenol (RBP) was observed in DT4 (82.3 and 2.3%) and DT5 (82.6 and 2.4%), followed by DT3 (75.4 and 1.8 %) and DT6 (75.5 and 1.7%) as their FSF and RBP values while DT2 (65.5 and 1.5%) had the lowest, respectively. Higher numerical values of free radicals for bound polyphenol (FSB) and reducing properties for soluble polyphenols (RFP) were recorded in DT5 (81.2 and 1.8%) and DT6 (81.1 and 1.8%), respectively (Table 4). This is followed by DT4 (76.6 and 1.6%), DT3 (63.4 and 1.6%, and DT2 (63.7 and 1.4%) as their FSB and RFP, respectively.

Table 5: Serum Enzyme Marker Levels of *C. gariepinus* fed Varying level of *S. indicum* inclusion

DT (%)	Enzyme				
	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)	LDH (U/L)
DT1	123.50±1.00^{a}	108.32±0.24^{a}	93.43±0.01^{a}	56.56±1.00^{a}	301.32±1.00^{a}
DT2	24.24±0.01^{e}	31.53±2.11^{e}	21.31±1.00^{e}	17.54±1.20^{e}	142.25±0.01^{f}
DT3	91.43±1.00^{c}	99.13±0.31^{b}	71.25±1.00^{c}	41.20±0.31^{c}	213.01±2.04^{b}
DT4	95.61±0.02^{b}	98.54±1.21^{c}	71.21±1.00^{c}	42.53±1.01^{b}	174.23±1.12^{d}
DT5	85.16±0.01^{d}	87.16±1.00^{d}	66.66±0.02^{d}	37.15±2.02^{d}	167.37±2.01^{e}
DT6	85.12±0.01^{d}	87.13±1.02^{d}	66.62±1.02^{d}	37,12±0.41^{d}	167.35±1.00^{e}
DT7	95.65±1.23^{b}	98.57±0.24^{c}	74.34±1.23^{b}	42.54±2.01^{b}	176.21±1.23^{c}

Data are presented as mean±standard deviation. Means with the different superscript letters within a column are significantly different ($p<0.05$). DT: dietary treatment, ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase, GGT: gamma-glutamyl transferase, LDH: lactate dehydrogenase, DT1: commercial diet with paracetamol (negative control), DT2: commercial diet only (control), DT3: 0% sesame seed meal, 100% soya bean meal and paracetamol, DT4: 25% sesame seed meal, 75% soya bean meal and paracetamol, DT5: 50% sesame seed meal, 50% soya bean meal and paracetamol, DT6: 75% sesame seed meal, 25% soya bean meal and paracetamol, DT7: 100% sesame seed meal, 0% soya bean meal and paracetamol.

Table 5 presented the results of liver marker enzymes of the fish fed diets with graded levels of *S. indicum* inclusion. It reveals that the serum ALT, AST, ALP, GGT and LDH contents of fish fed the control diet (DT1) and orally administered paracetamol were drastically increased compared to that of the group fed the control diet (DT2) only. In a surprise, those fish fed formulated diets with various graded levels of *S. indicum* embedded and orally administered acetaminophen has their serum marker enzyme levels significantly ($p<0.05$) lower than the group fed the control diet (DT1) and co-administration of acetaminophen (Table 5). All fishes served with DT5 and DT6 during the period of the experiment have observed a significant lower of liver enzyme marker levels than others (Table 5).

Table 6: Serum Chemistry Levels of *C. gariepinus* fed varying level of *S. indicum* inclusion

DT (%)	GLU (mg/dL)	TPN (g/L)	BIL (mg/dL)	CHL (mg/dL)	ALB (mg/dL)	CTN (mg/dL)
DT1	145.34 ± 1.00^{a}	4.23 ± 0.01^{g}	1.76 ± 0.01^{a}	254.12 ± 1.00^{a}	1.76 ± 1.00^{f}	2.64 ± 1.12^{a}
DT2	85.23 ± 0.01^{f}	6.51 ± 0.01^{f}	0.52 ± 1.20^{f}	93.01 ± 1.20^{e}	4.73 ± 0.01^{a}	0.54 ± 2.01^{e}
DT3	128.12 ± 1.00^{b}	7.52 ± 0.01^{e}	1.32 ± 1.02^{b}	172.03 ± 0.01^{b}	3.45 ± 0.01^{e}	1.23 ± 2.10^{b}
DT4	92.34 ± 0.02^{d}	8.01 ± 0.01^{d}	0.86 ± 1.00^{c}	162.24 ± 0.01^{c}	3.54 ± 0.01^{d}	0.94 ± 1.23^{c}
DT5	92.31 ± 0.01^{d}	9.56 ± 1.00^{a}	0.84 ± 2.02^{d}	147.26 ± 0.02^{d}	3.95 ± 0.01^{b}	0.76 ± 1.02^{d}
DT6	89.37 ± 0.01^{e}	9.14 ± 1.00^{b}	0.78 ± 2.02^{e}	147.23 ± 0.01^{d}	3.96 ± 1.00^{b}	0.77 ± 2.01^{d}
DT7	98.34 ± 2.01^{c}	8.56 ± 1.01^{c}	0.85 ± 2.13^{d}	162.21 ± 1.27^{c}	3.76 ± 1.34^{c}	0.92 ± 1.24^{c}

Data are presented as mean±standard deviation. Means with the different superscript letters within a column are significantly different ($p<0.05$). DT: dietary treatment, GLU: glucose, TPN: total protein, BIL: bilirubin, CHL: cholesterol, ALB: albumin, CTN: creatinine, DT1: commercial diet with paracetamol (negative control), DT2: commercial diet only (control), DT3: 0% sesame seed meal, 100% soya bean meal and paracetamol, DT4: 25% sesame seed meal, 75% soya bean meal and paracetamol, DT5: 50% sesame seed meal, 50% soya bean meal and paracetamol, DT6: 75% sesame seed meal, 25% soya bean meal and paracetamol, DT7: 100% sesame seed meal, 0% soya bean meal and paracetamol.

The results of the serum chemistry of fish fed diets with various percentages of *S. indicum* inclusion are shown in Table 6. It reveals that all the levels of serum biochemical indices studies in fish fed the control diet and acetaminophen intubation (DT1) were significantly (0<0.05) higher than those fish fed the control diet (DT2). However, the total protein (4.23g/L) and albumin (1.76 mg/dL) levels of fish fed control diet and acetaminophen orally administered were significantly ($P<0.05$) lower as compared to those of fish (6.51g/L and 4.73mg/dL), respectively, fed only control diet (DT2) (Table 6). Furthermore, fishes fed formulated diets incorporated with various levels of *S. indicum* and acetaminophen intubation recorded significant ($p<0.05$) lower in glucose, bilirubin, cholesterol, and creatinine levels than those fed the control diet (DT1) and acetaminophen orally given. Inversely, a significant ($p<0.05$) increased in total protein and albumin levels was noted in fish fed formulated diets with various graded levels of *S. indicum* inclusion and acetaminophen intubation than to those fed the control diet (DT2) with acetaminophen intubation (Table 6).

4. Discussion

The results of total phenol contents of formulated diets embedded with different levels of *S. indicum* as shown in Table 4 indicated that higher numerical values of total phenol contents were recorded in all formulated diets than to the control diet (DT1). It could be a reflection of the inclusion of varying graded levels of *S. indicum* inclusion in the formulated diets. This finding is supported by what Adeniyan *et al.* **[16]**, Jeong *et al.* [24] reported. Phenols are good antioxidant compounds with several effects on health improvement. They are bioactive compounds that have stable radical, potential to donate an electron, and preventing the oxidation of food ingredients. The ability of phenolic compounds to prevent oxidation of food ingredients reflected their redox potential, which has a significant role to play in neutralizing free radical species [4, 16].

Elevated values for free radical scavenging ability for soluble polyphenols (FSF), bound polyphenols (FSB) and reducing power of free soluble polyphenols (RFP) and bound polyphenols (RBP) were noted in all diets with varying levels of *S. indicum* than that of control diet (DT1) could be attributed to the increasing levels of *S. indicum* inclusion. It could be also signified the antioxidant potential of the diets due to the presence of *S. indicum*. These findings agreed with what was reported by Ndatsu *et al.* [4] and Adeniyan *et al.* **[16]**. Higher values of both free soluble and bound polyphenols in *S. indicum* extractions are documented [24]. The polyphenols (phenol, flavonoid) are potent antioxidants of plant origin. It does limit the bioavailability of reactive metals (zinc, iron, copper) by establishing complexes formation [25]. Metal ions (cations) bioavailability can also be retarded and the excess levels of these metals geared up the production of free radical species to cause oxidative stress-related damage in the cellular tissues [25]. Oxidative stress is the formation of an imbalance between the generation of free radical species and the system of antioxidant defense mechanisms [4].

Early signs of intensive disorder in the aquaculture system under stress can be determined using the assessment of the biochemical indices **[26]**. Aquaculture farming under stressful conditions that altered serum marker enzyme levels in fish has been documented. Increased levels of ALT and AST in Stressed fish could probably suggest the use of dietary amino-acids for growth as well as compensatory for energy demand as a response to the stressor. The levels of these enzymes in the blood serum are a reflection of hepatocyte states, which signify hepatocellular injury [27]. As indicated in Table 5, feeding fish with control diets (DT1) and co-administration of the mega dose of 100mg/g/day acetaminophen for four (4) weeks only, significantly ($p<0.05$) elevated the serum marker enzymes (ALT, AST, ALP, GGT, and LDH) than those fed control diet (DT2) alone (Table 5). Elevation in ALT and AST levels are signs of hepatic cell problem. It could be suggested that the high doses of acetaminophen applied causes the free radicals generation and eventually led to fish hepatocytes problem. Also, higher levels of these enzymes in the serum of fish could suggest oxidative stress production due to mega doses of acetaminophen administered orally.

Ndatsu *et al*. [4] have reported that increased levels of serum marker enzymes signify hepatic cell damage, which agreed with what Adesina, [28] reported that increased levels of Aminotransferase in the blood serum of fish are a response to stress conditions. That is high values of AST and ALT in the fish serum is a reflection of dying or hepatocyte injuries. Aminotransferase leakage from injured hepatic cells into the blood via hepatocytes damage [27]. In furtherance, significant elevation in serum levels of ALP and GGT in fish fed the control diet (DT1) and daily intubation of acetaminophen than those fed control diet (DT2) only could suggest cholestatic or infiltrative disorders in the fish hepatic cells caused by mega doses of acetaminophen intubated. Increase levels of ALP and GGT in the blood serum suggested cholestatic disorder and they are markers of cholestasis. This condition is referring to a stoppage or slow flow of bile from the liver, which obstructs the biliary system [27]. In a normal intake of acetaminophen, the cytochrome P-450 activated the production of excessive metabolites that can be reduced through glutathione. Therefore, if an overdose of acetaminophen is taken and the stored glutathione depleted, the excess metabolites generated reacted with the macromolecules of the hepatic cells and cause hepatocellular injury. This signifies that hepatic toxicity by mega doses of acetaminophen depends on cellular glutathione depletion [29]. Inversely, all fish fed formulated diets embedded with varying grades of *S. indicum* alongside megadose of acetaminophen recorded significant decrease $p<0.05$) in serum marker enzymes (ALT, AST, ALP, and GGT) compared to those fed normal di*et al*one (DT2). This finding might suggest the hepatoprotective capacity of the formulated diets against tissue oxidation produced by mega-dosing fish with acetaminophen. That is the mechanism of antioxidants could be involved in protecting hepatocytes by the formulated diets against drug-induced oxidative stress. Adeniyan *et al*. **[16]** and Jeong *et al*. **[24]** reported that *S. indicum* products contain higher antioxidant properties, which are capable of quenching and scavenging the free radicals in the macromolecular cells. Also, stuff foods are reported to be rich in many polyphenol compounds (phenols and flavonoids). The antioxidant capacity of flavonoids is documented to be stronger than those of vitamin C and E **[30]**. Lower levels of serum marker enzymes were recorded in *C. gariepinus* upon exposure to dietary intoxicants. The decrease of these enzyme activities could reflect their inhibition in the rate of production in the hepatic cells [16]. Subsequently, the level of serum LDH of *C. gariepinus* fed control diets with intubated acetaminophen (DT1) was elevated significantly ($p<0.05$) than those fed the control diet (DT2) only (Table 5). This finding suggests probably tissue damage produced by the acetaminophen-induced oxidative stress. That is a sign of hepatic dysfunction caused by free radicals generation upon acetaminophen-induced oxidative stress. The hepatic toxicity caused by acetaminophen can alter the membrane of liver microsomal through affected polyunsaturated fatty acids [4]. Elarabany and Bahnasawy [30] have reported that elevated LDH was discovered in lead-exposed fish and the increased levels of enzyme experienced might signify the generation of free radicals caused by acetaminophen overdose. LDH is an enzyme of metabolism used as a biomarker in the oxidatively stressed fish and its elevation above the normal range signifies the case of hepatocytes damage in heavy metal-induced oxidative stress [26, 31]. Malachy *et al*. **[32]** reported that elevated value of LDH was recorded in diclofenac (DCF)-exposed fish. Increased value of LDH was observed in heavy metal-induced stressed *C. gariepinus* [32]. Inversely, significantly decreased ($P<0.05$) of LDH values were noted in *C. gariepinus* fed formulated diets with varying grades of *S. indicum* and alongside acetaminophen intubation than those fed normal diets (DT2) alone (Table 5). These drastic reductions of LHD levels could signify a protective potential by formulated diets against hepatocytes injury caused by acetaminophen overdose. Decrement of LDH levels upon dietary treatment is a sign of mechanism of protection over the increased generation of reactive oxygen species (ROS) in stressed *C. gariepinus* [31].

As presented in Table 6, all *C. gariepinus* treated with the control diet with intubated acetaminophen (DT1) had their serum glucose, bilirubin, cholesterol, and creatinine levels significantly higher ($p<0.05$) than those fed control diet (DT2). This could be attributed to the ability of *C. gariepinus* to generate energy from the available means against the oxidative stress caused by acetaminophen. That is enough energy must be acquired by fish from the available sources to fight against the ROS generated. Invariably, the results of this study are relatively higher than the values reported by Adesina, [28], Anene *et al*. [34]. Adesina, [28] have reported that adequate energy liberation from the available sources is required by stressed fish to survive. Elevated blood glucose ranged from 150 to 208 mg/dL was reported in *C. gariepinus* **[35]**. High demand for glycogen mobilization may signify an increase level of blood glucose and depletion of glycogen in the liver and muscle upon anti-dietary exposure **[28]**. Interestingly, decreased levels of blood glucose observed in all *C. gariepinus* fed formulated diets and acetaminophen intubation may suggest potent hepatoprotection by diets with varying grade levels of *S. indicum* against ROS generated by acetaminophen overdose. The hepatoprotection exhibited by formulated diets may signify their ability to trigger insulin secretion from pancreatic cells to enhance the process of glycogenesis. It was reported that increase production of insulin from the pancreatic tissue in exposed fish to high antioxidant diets improves the glycogenesis processes [36]. A higher level of serum bilirubin in *C. gariepinus* fed a control diet with acetaminophen intubation (DT1) may suggest a compensatory process against biliary gland damage due to cellular peroxidative changes. The bilirubin *in vivo* plays vital roles as a potent ROS neutralizer, antimutagen, and a protector of endogenous tissue **[37]**. Its low levels recorded in fish fed formulated diets with graded levels of *S. indicum* embedded could signify their potential hepatic protection. The synthesis of bile acid depends on the stability of the function of the biliary cell and endoplasmic reticulum [38].

Besides, the recorded low levels of total protein and albumin in *C. gariepinus* fed the control diet and acetaminophen orally administered (DT1) could be due to the presence of antinutrients. Adesina [28] has reported that low levels of protein and albumin in animals (fish) exposed to a toxic agents might suggest the hydration state and water equilibrium changes or inhibition of protein synthesis in the liver [4]. In the previous study, the depletion of protein and albumin in fish as a way forward to resist against abnormal metabolic processes has been reported [28]. Proteins are one of the major energy sources in fish that monitor blood glucose maintenance [4]. However, the elevated values of total protein and albumin in *C. gariepinus* fed formulated diets with substituted *S. indicum* at various levels and acetaminophen intubation suggest their hepatic damage repair potential by normalizing the process of protein synthesis. That is all formulated diets are potent hepatoprotective over hepatotoxic produced by mega doses of acetaminophen. The antioxidant potentials of these diets could contribute to their hepatic protection mechanism against ROS generated. The antioxidant potentials exhibited by the formulated diets may suggest the presence of phenols and flavonoids [39, 40].

Lastly, the lower levels of serum total cholesterol and creatinine recorded in *C. gariepinus* fed formulated diets and acetaminophen intubation compared to those fed control diet and acetaminophen intubation (DT1) could be associated with the hepatoprotective potential of all diets against generated ROS by acetaminophen overdose. Low levels of serum cholesterol recorded in all fish fed formulated diets suggest protection against hypercholesterolemia and atherosclerosis. This could be probably due to the high antioxidant potential of various levels of *S. indicum* inclusion in the diets. Lin *et al*. [39] have reported the high antioxidant potential of *S. indicum*. It was reported that phenols and flavonoids could protect membrane lipids from oxidation, and a major source of flavonoids is foodstuff [40]. High level of creatinine in fish served control diet plus acetaminophen intubation compared to fish served control diet and acetaminophen signifies impaired kidney function or kidney disease caused by ROS induced by acetaminophen overdose. As the kidneys become impaired for any reason, the creatinine level in the blood will rise due to poor clearance of creatinine by the kidneys. Abnormally high levels of creatinine thus warn of possible malfunction or failure of the kidneys [28]. However, *C. gariepinus* fed formulated diets with varying levels of *S. indicum* inclusion showed a low level of serum creatinine ranged from 0.77 – 1.23 mg/dL could signify their potential nephroprotective properties. That is creatinine was properly utilized by the muscle of the fish against oxidative stress produced by acetaminophen overdose. The high values of phenols and flavonoids in *S. indicum* (Okoronkwo *et al*., 2014) [40] could be probably responsible for kidney damage repair.

Conclusion

The findings of this work showed that all formulated diets with varying percentages of *S. indicum* inclusion demonstrated high antioxidant properties and are good in repairing macrocellular damage in stressful subjected fish. However, formulated diets (DT5 and DT6) with 50 and 75% *S. indicum* looked more promising in addressing the oxidative stress-related disorders generated in fish after exposure to acetaminophen overdose.

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References

1. Ali Y, Munir O, Vahit B. The antioxidant activity of leaves of *Cydonia vulgaris*. Turkish Journal of Medical Sciences. 2001; 31:23-27
2. Sun J, Chu YF, Wu X, Liu RH. Antioxidant and Antiproliferative activities of common fruits. Journal of Agricultural and Food Chemistry. 2002; 50(25):7449-7454
3. Chu YF, Sun J, Wu X, Liu RH. Antioxidant and antiproliferative activities of common vegetables. Journal of Agricultural and Food Chemistry. 2002; 50(23):6910-6916.
4. Ndatsu Y, Ganiyu O, Amuzat AO. Antioxidant and Hepatoprotective Properties of Tofu (*Curdle Soymilk*) against Acetaminophen-Induced Liver Damage in Rats. Biotechnology Research International. 2013, 7
5. Coussens LM, Werb Z. Inflammation and cancer. Nature. 2000; 420(6917):860-7
6. Ricciotti E, FitzGerald GA. Prostaglandins and Inflammation. Arteriosclerosis, Thrombosis, and Vascular Biology. 2011; 31(5):986-1000
7. Graham GG, Scott KF. Mechanism of action of Paracetamol. American Journal of Therapeutics. 2005; 12(1):46-55
8. Klinger-Gratz PP, Ralvenius WTE. Acetaminophen relieves inflammatory pain through CB1 cannabinoid receptors in the rostral ventromedial medulla. Journal of Neuroscience. 2018; 38:322-34
9. Mitchell SJ, Kane AE, Hilmer SN. Age-related changes in the hepatic pharmacology and toxicology of paracetamol. Current Gerontology and Geriatrics Research. 2011, 1-14
10. Eriksson LS, Broomé U, Kalin M, Lindholm M. Hepatotoxicity due to repeated intake of low doses of paracetamol. Journal of Internal Medicine. 1992; 231(5):567-570
11. Caparrotta TM, Antoine DJ, Dear JW. Are some people at increased risk of paracetamol-induced liver injury? Critical review of the literature. European Journal of Clinical Pharmacology. 2018; 74(2):147-60
12. Peters, H, Gloria FN, Ikpeme CE. Nutritional evaluation of breadfruit and beniseed composite flours. MOJ Food Process Technology. 2016; 2(6):194-199
13. Garbia HAA, Shehata AAY, Shahidi F. Effect of Processing on oxidative stability and lipid classes of Sesame oil. Food Resource International. 2000; 33(5):331-340.
14. Alobo AP. Effect of sesame seed flour on millet biscuit characteristics. Plant Food Human Nutrition. 2001; 56(2):195-202
15. Uaboi PU, Sobande AO, Okolie PN. Proximate composition of whole dehulled and fermented beniseed (*Sesame indicum*) with associated bacteria species. Nigerian Food Journal. 2008; 26(1):1-6.
16. Adeniyan OO. Effect of Boiling on the Nutritional Composition and Antioxidant Properties of Beniseed (*Sesamum indicum* L.). Food Science and Quality Management. 2013, 11
17. Ayoola AA. Replacement of Fishmeal with Alternative Protein Sources in Aquaculture Diets. *A thesis* submitted to the Graduate Faculty of North Carolina State University in partial fulfillment of the requirements for the Degree of Master of Science, 2010
18. Alatise PS, Ogundele O, Eyo AA, Oludunjoye F. Evaluation of Different Soybean-Based Diets on Growth and Nutrient Utilization of *Heterobranchus longifilis* in Aquaria Tanks. Proceedings of the Fisheries Society of Nigeria (FISON) Conference held at the University of Calabar, Calabar, Nigeria, 13-17 November, 2006, 255-262
19. Keke IR, Anene A. Length-Weight Relationship and Fecundity of *Clarias gariepinus* from River Niger. Journal of Aquatic Science. 2011; 26 (1):1-7.

20. Olapade OJ, George PQ. Nutritional Evaluation of Defatted Groundnut Cake meal with Amino acid as Protein Supplement in African Catfish (*Clarias gariepinus* Burchell, 1822) Juveniles Diet. Journal of Fisheries and Aquatic Science. 2019; 7:14
21. Kumar V, Makkar HPS, Becker K. Detoxified *Jatropha curcas* kernel meal as a dietary protein source: Growth performance, nutrient utilization and digestive enzymes in common carp, *Cyprinus carpio* fingerlings. Aquaculture Nutrition. 2011; 7:313-326.
22. NIH Publication no. 85-23. Respect for life, National Institute of Environmental Health Sciences-NIEHS. 1985
23. Effiong MU, Akpan AW, Essien-Ibok MA. Effects of Dietary Protein Levels on Proximate, Haematological and Leukocyte Compositions of *Clarias gariepinus*. Journal of Applied Sciences and Environmental Management. 2019; 23(11):2065-2069
24. Jeong SM, Kim SY, Kim DR, Nam KC, Ahn DU, Lee SC. Effect of Seed Roasting Conditions on the Antioxidant Activity of Defatted Sesame Meal Extracts. Journal of food Science. 2004; 69(5):377
25. Nwanna EE, Oboh G. Antioxidant and hepatoprotective properties of polyphenol extracts from *Telfairia occidentalis* (Fluted Pumpkin) leaves on acetaminophen induced liver damage. Pakistan Journal of Biological Sciences. 2007; 10(16):2682-2687
26. Saliu JK, Bawa-Allah KA. Toxicological effects of lead and zinc on the antioxidant enzyme activities of post-juvenile *Clarias gariepinus*. Research Environment and life Sciences. 2012; 2:21-26.
27. Dorcas IK, Solomon RJ. Calculation Of Liver Function Test In *Clarias Gariepinus* Collected From Three Commercial Fish Ponds. Nature and Science. 2014; 12(10):107
28. Adesina SA. Haematological and Serum Biochemical Profiles of *Clarias gariepinus* Juveniles Fed Diets Containing Different Inclusion Levels of Mechanically Extracted Sunflower (*Helianthus annuus*) Seed Meal. Applied Tropical Agriculture. 2017; 22(2):24-35
29. Arnaiz SL, Llesuy S, Cutrin JC, Boveris A. Oxidative stress by acute acetaminophen administration in mouse liver. Free Radical Biology and Medicine. 1995; 19(3):303-310.
30. Al´ıa MC, Horcajo LB, Goya L. Effect of grape antioxidant dietary fiber on the total antioxidant capacity and the activity of liver antioxidant enzymes in rats. Nutrition Research. 2003; 23(9):1251-1267
31. Elarabany N, Bahnasawy M. Comparative and Interactive Biochemical Effects of Sub-Lethal Concentrations of Cadmium and Lead on Some Tissues of the African Catfish (*Clarias gariepinus*). Toxicological Research. 2019; 35:249-255
32. Malachy NOA, Ogo AO, Bala SA, Kyrian CU. Chronic diclofenac (DCF) exposure alters both enzymatic and haematological profile of African catfish, *Clarias gariepinus*. Drug and Chemical Toxicology. 2015; 38(4):383-390,
33. Walaa EH, Alshimaa AK, Yasmina MA, Haitham AB. The potential role of turmeric and black pepper powder diet supplements in reversing cadmium-induced growth retardation, ATP depletion, hepatorenal damage, and testicular toxicity in *Clarias gariepinus*. Aquaculture. 2019; 510:109-121
34. Anene A, Ukpabi UH, Ibewuike J, Jumbo VN. Some haematological responses of fingerlings of *Clarias gariepinus* fed different dietary levels of boiled *Mucuna cochichinensis* seed meal. Journal of Aquaculture Feed Science and Nutrition. 2014; 6(2):51-58
35. Yilmaz E, Akyurt I, Mutlu E. Effects of energetic diets on growth, blood chemistry and liver pathology of African catfish *Clarias gariepinus* (Burchell 1822). Israeli Journal of Aquaculture, Bamidgeh. 2006; 58:191-197.
36. Gbore FA, Oginni O, Adewole AM, Aladenton JO. The effect of transportation and handling stress on haematology and plasma biochemistry in fingerlings of *Clarias gariepinus* and *Tilapia zillii*. World Journal of Agricultural Science. 2006; 2:208-212
37. Pratibha K, Anand U, Agarwal R. Serum adenosine deaminase, 5' nucleotidase and malondialdehyde in acute infective hepatitis. Indian Journal of Clinical Biochemistry. 2004; 19(2):128-131
38. Sureshkumar SV, Mishra SH. Hepatoprotective activity of extracts from *Pergularia daemia* Forsk. against carbon tetrachloride induced toxicity in rats. Pharmacognosy Magazine. 2007; 3(11):187-191
39. Lin Z, Xiaohui L, Arshad MA, Bisheng Z. Phytochemical Contents and Antioxidant and Antiproliferative Activities of Selected Black and White Sesame Seeds. BioMed Research International. 2016, 9
40. Okoronkwo NE, Osuji EQ, Nwankwo CC. Assessment of potential values of *Sesamum indicum* seed. International Journal of Arts & Sciences. 2014; 7(3):19-27.