

Research Article

Effect of *Curcuma longa* Rhizome Powder on Metabolic Parameters and Oxidative Stress Markers in High-Fructose and High-Fat Diet-Fed Rats

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Received 26 June 2023; Revised 18 December 2023; Accepted 28 December 2023; Published 6 January 2024

Academic Editor: Tanmay Sarkar

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Metabolic disorders have become a major and growing global health problem, so finding potentially novel solutions with fewer harm is favourable to solving this problem. Thus, this research aimed to determine the effect of a diet supplemented with Curcuma longa rhizome powder on markers of oxidative stress as well as biochemical and haematological parameters of rats with dietinduced metabolic disorders. The powder was obtained from fresh Curcuma longa rhizome for preparation of extracts, which were used for in vitro analysis. After induction of metabolic disorders in male Wistar rats, they were separated into five groups: base, control, and high-fructose and high-fat diet supplemented with Curcuma longa rhizome powder at 2.5%, 5%, and 10%, respectively. The phytochemical analysis of the Curcuma longa rhizome powder extracts revealed the presence of phenols (96.97 ± 0.79 mg GAE/g) and flavonoids (133.19 ± 1.64 mg CE/g) and also showed good activities in DPPH free radical scavenging (79.46 ± 2.12%) and good inhibition of alpha-amylase enzyme (94.12 ± 2.84%). The food supplementation with Curcuma longa rhizome powder lowered Lee index (326.88 ± 3.08 to 262.45 ± 4.71), glycaemia (189.25 ± 5.98 to 120.5 ± 4.91 mg/dl), peritoneal fat deposition $(2.18 \pm 0.02 \text{ to } 1.35 \pm 0.14 \text{ g}/100 \text{ g of body weight})$, triglycerides $(58.35 \pm 5.37 \text{ to } 109.94 \pm 1.42 \text{ mg/dl})$, total cholesterol $(208.53 \pm 1.53 \text{ to } 114.18 \pm 2.20 \text{ mg/dl})$, LDL-cholesterol $(128.73 \pm 3.64 \text{ to } 17.55 \pm 1.59 \text{ mg/dl})$, malondialdehyde $(67.54 \pm 2.48 \text{ to } 17.55 \pm 1.59 \text{ mg/dl})$, malondialdehyde $(67.54 \pm 2.48 \text{ to } 17.55 \pm 1.59 \text{ mg/dl})$, malondialdehyde $(67.54 \pm 2.48 \text{ to } 17.55 \pm 1.59 \text{ mg/dl})$, malondialdehyde $(67.54 \pm 2.48 \text{ to } 17.55 \pm 1.59 \text{ mg/dl})$, malondialdehyde $(67.54 \pm 2.48 \text{ to } 17.55 \pm 1.59 \text{ mg/dl})$, malondialdehyde $(67.54 \pm 2.48 \text{ to } 17.55 \pm 1.59 \text{ mg/dl})$, malondialdehyde $(67.54 \pm 2.48 \text{ to } 17.55 \pm 1.59 \text{ mg/dl})$, malondialdehyde $(67.54 \pm 2.48 \text{ to } 17.55 \pm 1.59 \text{ mg/dl})$, malondialdehyde $(67.54 \pm 2.48 \text{ to } 17.55 \pm 1.59 \text{ mg/dl})$, malondialdehyde $(67.54 \pm 2.48 \text{ to } 17.55 \pm 1.59 \text{ mg/dl})$, malondialdehyde $(67.54 \pm 2.48 \text{ to } 17.55 \pm 1.59 \text{ mg/dl})$, malondialdehyde $(67.54 \pm 2.48 \text{ to } 17.55 \pm 1.59 \text{ mg/dl})$, malondialdehyde $(67.54 \pm 2.48 \text{ to } 17.55 \pm 1.59 \text{ mg/dl})$, malondialdehyde $(67.54 \pm 2.48 \text{ to } 10.59 \text{ mg/dl})$, malondialdehyde $(67.54 \pm 2.48 \text{ to } 10.59 \text{ mg/dl})$, malondialdehyde $(67.54 \pm 2.48 \text{ to } 10.59 \text{ mg/dl})$, malondialdehyde $(67.54 \pm 2.48 \text{ to } 10.59 \text{ mg/dl})$, malondialdehyde $(67.54 \pm 2.48 \text{ to } 10.59 \text{ mg/dl})$, malondialdehyde $(67.54 \pm 2.48 \text{ to } 10.59 \text{ mg/dl})$, malondialdehyde $(67.54 \pm 2.48 \text{ to } 10.59 \text{ mg/dl})$, malondialdehyde $(67.54 \pm 2.48 \text{ to } 10.59 \text{ mg/dl})$, malondialdehyde $(67.54 \pm 2.48 \text{ to } 10.59 \text{ mg/dl})$, malondialdehyde $(67.54 \pm 2.48 \text{ to } 10.59 \text{ mg/dl})$, malondialdehyde $(67.54 \pm 2.48 \text{ to } 10.59 \text{ mg/dl})$, malondialdehyde $(67.54 \pm 2.48 \text{ to } 10.59 \text{ mg/dl})$, malondialdehyde $(67.54 \pm 2.48 \text{ to } 10.59 \text{ mg/dl})$, malondialdehyde $(67.54 \pm 2.48 \text{ to } 10.59 \text{ mg/dl})$, malondialdehyde $(67.54 \pm 2.48 \text{ to } 10.59 \text{ mg/dl})$, malondialdehyde $(67.54 \pm 2.48 \text{ to } 10.59 \text{ mg/dl})$, malondialdehyde $(67.54 \pm 2.48 \text{ to } 10.59 \text{ mg/dl})$, malondialdehyde $(67.54 \pm 2.48 \text{ to } 10.59 \text{ mg/dl})$, malondialdehyde $(67.54 \pm 2.48 \text{ to }$ 39.75 ± 1.02 nmol/ml), and nitric oxide (27.83 ± 1.44 to 19.36 ± 1.28 nmol/ml) and increased HDL-cholesterol (48.12 ± 1.82 to 74.21 ± 1.04 mg/dl) and catalase activity (4.12 ± 0.56 to 11.58 ± 0.83 U/min/ml) with the best result at 5% supplementation. According to these results, the Curcuma longa rhizome powder supplement is a promising alternative for the treatment of metabolic disorders and oxidative stress.

1. Introduction

Environmental and behavioural changes such as the adoption of a westernised diet (high energy intake) and sedentary habits due to socioeconomic development are recognised as the main causes of metabolic disorders [1]. This complex disease state is defined by the occurrence of type 2 diabetes (insulin resistance), obesity, atherogenic hypertension, and dyslipidaemia that predisposes the population to an increased risk of developing cardiovascular diseases [1, 2]. These disorders are characterised by an increase in oxidative processes, promoting and aggravating other metabolic conditions, thus increasing death worldwide [3]. Based on the diagnostic criteria, the world prevalence of these disorders varies from 13.6% to 46.0% [4]. Improving means used to prevent risk factors include drug treatment and adjuvant therapy [5]. However, the low effectiveness, high cost, and adverse effects of the latter have prompted the quest for the search of natural alternatives in the form of natural products, functional foods, or nutraceuticals [5].

Among these alternatives, spices have been recognised as high potential sources of renewable and biodegradable chemicals that are efficient in preventing and/or treating metabolic disorders. Indeed, spices are gaining interest due to anti-inflammatory, carminative, antioxidant, and antiseptic properties, thanks to which their use in producing nutraceuticals or functional foods has been enhanced [6].

Curcuma longa, also referred to as turmeric, belongs to the family Zingiberaceae and has a golden colour with a slightly bitter taste. It is mostly produced in India and is also present in the Cameroonian flora. It is considered as an important spice used in most culinary preparations worldwide [5, 6]. The phytochemical compounds of this plant are present in the rhizome, made up of 77% curcumin, 17% bisdemethoxycurcumin, and 3% demethoxycurcumin [7-10]. Several research works had shown the beneficial effect of Curcuma longa in the treatment of various pathological states due to its antiinflammatory [11, 12], antioxidant [13], antimicrobial, antibacterial, antiviral [14, 15], antidiabetic [16], and antihypertension [17, 18] properties. Curcumin competitively inhibits α -amylase and reduces blood glucose levels in diabetic rats [16, 19, 20]. It also has protective properties against teratogenic, cytotoxic, and neurodegenerative diseases [21, 22] and hepatoprotective [23] and nephroprotective activities against hepato-renal failure [24, 25]. To benefit from the health properties of this spice, it is consumed in the form of powder added to food during cooking, essential oils (either as nutraceutical or supplemented with food), and nanoparticles [6, 23]. The essential oils as well as nanoparticles are not affordable for LMICs due to their high production costs. These populations mainly rely on the culinary powder form without information on the proportion that can efficiently provide the health benefits awaited. This work was thus aimed to assess the effect of diet supplementation with various proportions of Curcuma longa rhizome powder on metabolic parameters and oxidative stress markers of rat-induced metabolic disorders.

2. Materials and Methods

2.1. Plant Material Sampling. Curcuma longa rhizomes were harvested in Santchou (Menoua Division, West Cameroon) and transported to the Research Unit of Biochemistry, Medicinal Plants, Food Sciences and Nutrition (URBP-MAN) for further studies. Thereafter, the plant identification was done at the National Herbarium of Cameroon and the reference number 42173/HNC was assigned.

2.2. Preparation of Powder. Fresh rhizomes of Curcuma longa were cleaned, sliced, and oven-dried (Venticell, Turkey) at 45°C for 48 hours. The dried rhizomes were

later ground using a blender (Singsung, model no: BL-500, Singapore) to obtain the *Curcuma longa* rhizome powder.

2.3. Preparation of Extract. The extracts were prepared following the method reported by Womeni et al. [26]. 25 g of powder was introduced in three different beakers containing, respectively, 500 ml of distilled water, 500 ml of ethanol 90%, and 500 ml of mixed ethanol and distilled water (50% V/V); the beakers were then manually shaken every 3 hours during 48 h storage at room temperature (25°C) in order to obtain aqueous, hydroethanolic, and ethanolic extracts. The mixtures were filtered using a Whatman paper (No. 1), and the filtrates were subsequently oven-dried at 45°C until complete evaporation of the solvent. The resulting extracts were stored at 4°C, protected from light.

2.4. Total Phenolic Content (TPC) Assessment. The total phenolic content (TPC) was determined using the modified method of Gao et al. [27]. In brief, $20 \,\mu$ l of extracts (2 mg/ml) was mixed, respectively, with 2 ml of distilled water and $200 \,\mu$ l of Folin–Ciocalteu reagent, and the mixture was incubated for 3 min at room temperature. Thereafter, the mixture received 1 ml of sodium carbonate and was reincubated for 2 hours at room temperature, and the absorbance of the resulting blue colour was measured at 765 nm using a spectrophotometer (BIOMATE, France). For standard curve preparation, a 0.2 g/l gallic acid solution was used, and the results were expressed as mg gallic acid equivalents per gram of powder extract (GAE/g).

2.5. Total Flavonoid Assessment (TFC). The total flavonoid content (FC) was assessed thanks to the method of Marinova et al. [28]. Thus, $100 \,\mu$ l of *Curcuma longa* rhizome powder extracts (2 mg/ml) was mixed with 1.4 ml of distilled water and $30 \,\mu$ l of sodium nitrate (5% m/v). After 5 min, $200 \,\mu$ l of aluminium trichloride (10% w/V) was added, followed by the addition of $200 \,\mu$ l of sodium hydroxide (10% w/V) and $240 \,\mu$ l of distilled water after another 5 min. The mixture was then shaken, and the absorbance was measured at 510 nm using a spectrophotometer (BIOMATE, France). For standard curve preparation, a 0.1 mg/ml solution of catechin was used, and the results were expressed as mg catechin equivalents per gram of powder extract (CAE/g).

2.6. Evaluation of DPPH Free Radical Scavenging. The DPPH free radical scavenging activity of the rhizomes powder extracts was evaluated using the method of Mensor et al. [29]. In this experiment, 1 ml of a 0.3 mM methanolic solution of DPPH was mixed with 2.5 ml of each extract at increasing concentrations (12.5, 25, 50, 100, and $200 \mu g/ml$). These solutions were held in the dark at room temperature for 30 min, after which, the optical density (OD) was measured at 517 nm. A 2 mg/ml vitamin C solution was used as a positive control. The radical scavenging activity (RSA) was evaluated using the following formula:

$$RSA(\%) = \frac{OD \text{ of } DPPH - OD \text{ of sample}}{OD \text{ of } DPPH} \times 100.$$
(1)

2.7. Ferric Reducing Antioxidant Power (FRAP) Assessment. To determine the ferric reducing antioxidant power (FRAP) of powder extracts, their activity to reduce Fe³⁺ to Fe²⁺ was evaluated as described by Oyaizu [30]. In glass tubes containing 0.5 ml of powder extracts at increasing concentrations (12.5, 25, 50, 100, and 200 µg/ml), were added 1 ml of 0.2 M phosphate buffered saline at pH 6.6 and 1 ml of 1% of aqueous potassium hexacyanoferrate solution. After incubating the mixture at 50°C for 30 min, 1 ml of 10% trichloroacetic acid was introduced, followed by centrifugation for 10 min at $3000 \times g$. The supernatant (1.5 ml) was removed and mixed with 1.5 ml of distilled water as well as 0.1 ml of iron trichloride aqueous solution 0.1% (w/V), and the absorbance of the mixture was measured at 700 nm. Vitamin C (2 mg/ml) prepared under the same conditions was used as control. An increase in the absorbance of the mixture attested a rise in reducing the power of the powder extracts to reduce Fe^{3+} to Fe^{2+} .

2.8. Alpha-Amylase Inhibition Assay. The alpha-amylase inhibition assay was performed using the method reported by Manikandan et al. [31]. The starch solution (0.1% w/V) was obtained by adding 0.1 g of starch to 100 ml of sodium acetate buffer (16 mM). Then, the enzyme solution was obtained by dissolving 27.5 mg of alpha-amylase in 100 ml of distilled water. To prepare the colorimetric reagent, 3,5-dinitrosalicylic acid (96 mM) and sodium potassium tartrate solutions were mixed together. Subsequently, the control and powder extracts were added to the starch solution and allowed to react with the alphaamylase solution under alkaline conditions at 37°C and the reaction was measured over 15 min. The reaction products released were measured using a spectrophotometer at 540 nm.

2.9. Experimental Animals and Diet. Twenty Wistar male rats aged 4 weeks and weighing 65-75 g were obtained from the Animal Reproduction Unit of the Department of Biochemistry, University of Dschang. They were then housed in individual cases in an air-conditioned room (22–25°C) with a 12h light/dark cycle and had free access to food and fructose (Qualikems, Product no: F024112) solution ad libitum. This study was carried out with due regard to the welfare of animals, as per the recommendation of the Ethical Review Committee of the Faculty of Science (University of Dschang-Cameroon) and was designed following the internationally accepted standard ethical guidelines for laboratory animals' use and care prescribed by the European Union Institutional Ethics Committee on Animal Care (Council EEC 86/609/EEC of the 24th November 1986). The report on this section adheres to the guidelines for reporting animal research [32].

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The modified base diet proposed by Telefo et al. [33] was used in this study. The high-fat diet was prepared in the laboratory by mixing the powdered ingredients and fat (beef suet) as shown in Table 1.

2.10. Experimental Design and Collection of Blood and Organs. The experimental rats were divided into five groups of 4 rats each, and each rat was fed with 45 g of diet per day. The induction of metabolic disorder in rats and the administration of *Curcuma longa* powder supplementation in order to regulate metabolic parameters lasted for 12 and 4 weeks, respectively.

- (a) Group 1 (base group): received basal diet and pure water.
- (b) Group 2 (control group): received a high-fructose and high-fat diet.
- (c) Group 3: received a high-fructose and high-fat diet supplemented with 2.5% of *Curcuma longa* rhizome powder.
- (d) Group 4: received a high-fructose and high-fat diet supplemented with 5% of *Curcuma longa* rhizome powder.
- (e) Group 5: received a high-fructose and high-fat diet supplemented with 10% of *Curcuma longa* rhizome powder.

During the supplementation period, the weekly body weight and food intake were recorded. After a four week treatment period, the animals were allowed to fast for 12 h, followed by anaesthetisation with diazepam/ketamine (0.2:0.1 ml/100 g of animal), euthanisation, and blood collection by cardiac puncture in dry and EDTA tubes for haematological analyses. The blood samples in dry tubes were centrifuged at 3000 g for 15 min at 4°C, 3 hours following their collection. Separated serum was transferred into cryotubes and stored at -20°C for further analysis. Liver, peritoneal, and mesenteric fats were immediately collected and weighed. One gram (1 g) of liver tissue was homogenised in 10 ml of phosphate buffer (pH 7.5), centrifuged at 3000 g, and used for *in vivo* antioxidant tests.

2.11. Animal's Glycaemia and Lee Index. The device used for the measurement of blood glucose was a commercial glucometer (VERI Q, MGD-2001, Germany). On VERI Q strips, we placed a drop of fresh blood from the tail of the rat, which was allowed for 45 seconds to read the blood glucose value from the beginning to the end of the experiment. Body weight as well as the length from the nose to the anus was measured to evaluate the Lee index, which permitted determining the state of obesity in rats. This index was calculated by the formula which determines the link between the weight and the length of the animal from the nose to the anus [34]. Day 0 (day before high-fructose high-fat diet administration) marked the start of recording parameters, while day 1 represented the day the groups fed high-fructose high-fat diet were confirmed with glycaemia ≥180 mg/dl and Lee index \geq 300 in order to verify the diagnoses of insulin resistance and obesity.

TABLE 1: Composition of the basal diet and high-fat diet (g/1000 g of food).

	Content (in g/1000 g of food)				
Ingredients	Basal diet	High-fat (30% fat) and high-fructose diet			
Maize flour	678	500			
Soy flour	200	160			
Fish powder	100	85			
Bone powder	10	10			
Table salt	10	10			
Beef suet	0	225			
Vegetable oil	1	0			
Vitamin complex	1	10			
Fructose solution	0	5%			

$$\mathbf{LI} = \left(\frac{\sqrt[3]{\mathbf{W}}}{\mathbf{L}}\right) \times \mathbf{1000},\tag{2}$$

where **W** is the body weight (g) and **L** is the length from the nose to the anus (cm).

2.12. Evaluation of Biochemical Parameters. The biochemical parameters evaluated in the serum were aspartate transaminase (AST), alanine transaminase (ALT), triglyceride, total cholesterol, and high-density lipoprotein cholesterol (HDLc) content following appropriate kits' (Spinreact, Santa Coloma, Spain) guides. To determine the low-density lipoprotein cholesterol (LDLc), the direct method proposed by Friedewald et al. [35] was used.

$$LDL = Total Cholesterol - \left(HDL + \frac{Triglyceride}{5}\right). \quad (3)$$

2.13. Determination of Nitric Oxide (NO) Concentration. The NO concentrations of samples were determined by the methods described by Tracey et al. [36]. For this purpose, $340 \,\mu$ l of sample was introduced into the tubes, then $340 \,\mu$ l of sulfanilamide (1% w/V) prepared in orthophosphoric acid (1% V/V) was added, and the mixtures were kept in the dark for 5 min at room temperature. Then, $340 \,\mu$ l of naphthylenediamine (NED, 1% w/V) was added to the reaction medium, and the whole solution was once more left in the dark for 5 min at room temperature. The optical densities of samples were measured at 520 nm. The nitric oxide concentration (NO) was determined from a standard curve obtained from various concentrations of sodium nitrate (0.1 mM).

2.14. Determination of Malondialdehyde (MDA) Concentration. The MDA concentration of the samples was determined following the methods proposed by Niehaus and Samuelsson [37]. In tubes containing $100 \,\mu$ l of sample, $500 \,\mu$ l of thiobarbituric acid (TBA, $1\% \,V/V$) prepared in 1% trichloroacetic acid and $500 \,\mu$ l of phosphoric acid ($1\% \,V/V$) were added. The mixture was incubated in a water bath at 100° C for 15 min and then cooled. Subsequently, the tubes were centrifuged at 3000 g for 10 min at room temperature

 $(25^{\circ}C)$ and the absorbance of the resulting pink colour of the supernatant was measured at 532 nm. The blank contained 100 μ l of distilled water and was treated under the same conditions. The concentration of MDA was calculated using the following formula:

$$MDA = Ab x 1000 x Vt x \propto x V, \tag{4}$$

where Ab is the average absorbance, Vt is the total volume of the reaction medium, α is the molecular extinction coefficient (α : 1.53 × 105 M⁻¹ cm⁻¹), and *V* is the sample volume.

2.15. Determination of Catalase Activity. The catalase activity of the samples was assessed by their ability to reduce hydrogen peroxide (H₂O₂) to water (H₂O) and oxygen (O₂) as described by Sinha [38]. In tubes containing 50 μ l of sample, 750 μ l of phosphate buffer and 200 μ l of 50 mM hydrogen peroxide (H₂O₂) were added, followed by addition of 2 ml of 5% dichromate 1 min later. The tubes were rigorously shaken and heated for 10 min in boiling water. After cooling the tubes, the absorbance of the green colour generated was measured at 570 nm. The catalase activity (CAT) was determined using the following formula:

$$CAT = Ab x 1000 x Vt x \alpha x V,$$
 (5)

where Ab is the average absorbance, Vt is the total volume of the reaction medium, α is the molar extinction coefficient $(40 \text{ M}^{-1} \text{ cm}^{-1})$, and V is the sample volume.

2.16. Haematological Analysis. From EDTA tubes containing blood samples, blood cells were counted using an impedance haematology automatic counter (Shenzhen Mindray, BC-3600, China). The parameters analyzed included haematocrit (HCT), red blood cells (RBC), haemoglobin (HGB), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), white blood cells (WBC), erythrocytes (E), monocytes (MCT), lymphocytes (LYMP), and platelets (PLT).

2.17. Statistical Analysis. The results of the biochemical parameters, markers of oxidative stress, total phenolic and flavonoid contents, and haematological parameters were firstly expressed as mean \pm standard deviation. Then, they were analyzed using one-way analysis of variance (ANOVA), and Waller-Duncan post hoc test was used to compare the significance between the mean values using the IBM SPSS Statistics 22 (Chicago, IL, USA). Significance was considered for a *p* value <0.05. In addition, Pearson's correlation between total phenolic content (TPC), flavonoid content (FC), DPPH free radical scavenging (DPPH), ferric reducing antioxidant power (FRAP), and inhibition of alpha-amylase (IAA) was evaluated using SPSS version 22.

3. Results

3.1. Total Phenolic and Flavonoid Contents. Table 2 presents the total phenolic content (TPC) and flavonoid content (FC) of *Curcuma longa* rhizome extracts. It appears that TPC and

TABLE 2: Total phenolic content (TPC) and flavonoid content (FC) of *Curcuma longa* rhizome extracts.

Extracts	TPC (mg GAE/g)	FC (mg CE/g)
Aqueous	61.65 ± 1.58^{a}	44.21 ± 1.09^{a}
Hydroethanolic	133.19 ± 1.64^{c}	$96.97 \pm 0.79^{\circ}$
Ethanolic	74.55 ± 2.37^{b}	58.49 ± 1.90^{b}

Values (n = 3) with different superscript letters in the same row are significantly different (p < 0.05) according to Waller-Duncan post hoc test.

FC were significantly highest (p < 0.05) in hydroethanolic extract (133.19 ± 1.64 mg GAE/g and 96.97 ± 0.79 mg CE/g, respectively). The latter was followed by ethanolic extract (74.55 ± 2.37 mg GAE/g and 58.49 ± 1.90 mg CE/g, respectively) and aqueous extract being the lowest (61.65 ± 1.58 mg GAE/g and 44.21 ± 1.09 mg CE/g, respectively).

3.2. DPPH Free Radical Scavenging and Ferric Reducing Antioxidant Power. Table 3 illustrates the DPPH free radical scavenging and ferric reducing antioxidant power (FRAP) activities of *Curcuma longa* rhizome powder extracts. It can be observed that the antioxidant activities are significantly influenced (p < 0.05) by the concentration of the extracts. At 12.5 µg/ml, representing the lowest concentration tested, the hydroethanolic extract scavenged $34.95 \pm 2.05\%$ DPPH radical compared to $18.98 \pm 2.81\%$ for ethanolic extract and only $7.91 \pm 2.67\%$ for aqueous extract. At the maximum tested concentration (200μ g/ml), a strong DPPH scavenging activity ($79.46 \pm 2.12\%$) was evaluated in hydroethanolic extracts.

3.3. Inhibition of Alpha-Amylase. The inhibition of alphaamylase by the different extracts is grouped in Table 4. The *Curcuma longa* rhizome extracts revealed an important inhibitory action on the alpha-amylase enzyme. The inhibition significantly increased (p < 0.05) with the increasing concentration of *Curcuma longa* rhizome extracts from 250 to 1000 µg/ml. The hydroethanolic extract showed the best inhibition activity (94.12 ± 2.84%) on the enzyme, followed by the ethanolic extract ($62.52 \pm 3.71\%$) and the aqueous extract being the lowest ($42.19 \pm 3.43\%$) at the dose 1000μ g/ml.

3.4. Correlation between Total Phenolic Content (TPC), Flavonoid Content (FC), DPPH Free Radical Scavenging (DPPH), Ferric Reducing Antioxidant Power (FRAP), and Inhibition of Alpha-Amylase (IAA) of Curcuma longa Rhizome Powder Extracts. Table 5 presents Pearson's correlation between TPC, FC, DPPH, FRAP, and IAA. There was a significant positive correlation (r = 0.976, p < 0.01) between the TPC and FC. A significant positive correlation (r = 1.000, p < 0.01) was observed between the DPPH, TPC, and FC (r = 0.969). Another significant positive correlation (r = 1.000, p < 0.01) was observed between the FRAP, TPC, FC (r = 0.971), and DPPH (r = 1.000). Also, a significant positive correlation (r = 1.000, p < 0.01) existed between the IAA, TPC, FC (r = 0.969), DPPH (r = 1.000), and FRAP (r = 1.000). 3.5. Effect of the Curcuma longa Rhizome Powder Supplementation on the Lee Index of High-Fructose High-Fat Diet-Fed Rats. The Lee index of the rats in various groups is represented in Table 6. High-fructose high-fat diet-fed rats showed a significantly (p < 0.05) increased Lee index (326.88 ± 3.08) as compared to the base group (287.43 ± 4.32) after induction of metabolic disorder. Curcuma longa rhizome powder supplementation at 10% in high-fructose high-fat diet-fed rats significantly (p < 0.05) decreased the final Lee index (262.45 ± 4.71) compared to the control group (332.43 ± 6.94) after supplementation.

3.6. Effect of Curcuma longa Rhizome Powder Supplementation on the Glycaemia of the High-Fructose High-Fat Diet-Fed *Rats.* The glycaemia test was performed to evaluate the effect of consumption of Curcuma longa rhizomes powder supplementation on the fasting blood glucose after 28 days. The results of the glycaemia test are depicted in Table 7. At the start of the experiment, the initial blood glucose of the five (5) groups was approximately 127 mg/dl. The glucose concentration of the high-fructose high-fat diet-fed rats significantly (p < 0.05) increased $(189.25 \pm 5.98 \text{ mg/dl})$ compared to the base group $(134.5 \pm 3.10 \text{ mg/dl})$ after induction of metabolic disorder. Curcuma longa rhizome powder supplementation at 10% in high-fructose high-fat diet-fed rats significantly (p < 0.05) decreased the final blood glucose concentration $(120.5 \pm 4.91 \text{ mg/dl})$ compared to the control group $(186 \pm 6.94 \text{ mg/dl})$ after supplementation.

3.7. Effect of the Curcuma longa Rhizome Powder Supplementation on the Lipid Profiles of the High-Fructose High-Fat Diet-Fed Rats. Table 8 displays the lipid profile of rats. It can be highlighted that serum triglyceride, total cholesterol, and LDL-cholesterol levels were significantly (p < 0.05) increased in rats that consumed the high-fructose high-fat diet $(158.35 \pm 5.37 \text{ mg/dl}, 208.53 \pm 1.53 \text{ mg/dl}, \text{ and } 128.73 \pm$ 3.64 mg/dl, respectively) compared to the base group rats $(128.43 \pm 1.11 \text{ mg/dl}, 124.40 \pm 4.63 \text{ mg/dl}, \text{ and } 39.26 \pm 3.78$ mg/dl, respectively). Curcuma longa rhizome powder supplementation at 5% in high-fructose high-fat diet-fed rats significantly (p < 0.05) decreased the serum triglyceride, total cholesterol, and LDL-cholesterol level in high-fructose high-fat diet-fed rats $(116.41 \pm 2.65 \text{ mg/dl}, 119.94 \pm 3.86 \text{ mg/})$ dl, and 20.44 ± 3.61 mg/dl, respectively) compared to the control group ($158.35 \pm 5.37 \text{ mg/dl}$, $208.53 \pm 1.53 \text{ mg/dl}$, and 128.73 ± 3.64 mg/dl, respectively). Furthermore, the HDLcholesterol level significantly (p < 0.05) decreased in highfructose high-fat diet-fed rats (48.12 ± 1.82 mg/dl) compared to the base group rats ($59.44 \pm 1.70 \text{ mg/dl}$). Curcuma longa rhizome powder supplementation at 5% significantly (p < 0.05) increased the serum HDL-cholesterol level in high-fructose high-fat diet-fed rats $(74.21 \pm 1.04 \text{ mg/dl})$ compared to the control group rats $(48.12 \pm 1.82 \text{ mg/dl})$.

3.8. Effect of Curcuma longa Rhizome Powder Supplementation on Serum and Liver Levels of Oxidative Stress Markers in High-Fructose High-Fat Diet-Fed Rats. The results of the

 $64.20\pm1.73^{\rm e}$

 198.59 ± 0.86^{g}

 246.01 ± 2.70^{1}

Extracts	Concentration (µg/ml)	DPPH activity (%)	FRAP activity (μ mol Trolox/g)
	12.5	7.91 ± 2.67^{a}	7.50 ± 1.30^{a}
	25	$19.36 \pm 1.37^{\rm b}$	23.82 ± 1.62^{b}
Aqueous	50	$28.49 \pm 1.30^{\rm cd}$	51.41 ± 2.70^{d}
1	100	32.13 ± 1.37^{de}	$178.82 \pm 1.30^{\rm f}$
	200	$46.45 \pm 2.67^{\rm g}$	$211.08 \pm 2.27^{\rm h}$
	12.5	$34.95 \pm 2.05^{\rm e}$	$38.38 \pm 2.92^{\circ}$
	25	$41.50 \pm 2.67^{\rm f}$	$67.42 \pm 1.95^{\rm e}$
Hydroethanolic	50	$50.04 \pm 0.20^{ m gh}$	$197.21 \pm 2.60^{\rm g}$
•	100	69.66 ± 2.54^{j}	272.22 ± 1.40^{j}
	200	79.46 ± 2.12^{k}	301.72 ± 2.16^{k}
	12.5	$18.98 \pm 2.81^{\rm b}$	19.61 ± 2.81^{b}
	25	$24.41 \pm 1.99^{\circ}$	$38.15 \pm 1.73^{\circ}$

 $39.41 \pm 2.05^{\text{f}}$ $52.03 \pm 1.37^{\text{h}}$

 59.36 ± 1.44^{i}

TABLE 3: DPPH free radical scavenging and ferric reducing antioxidant power of Curcuma longa rhizome extracts.

Values (n = 3) with different superscript letters in the same row are significantly different (p < 0.05) according to Waller-Duncan post hoc test.

TABLE 4: In vitro alpha-amylase inhibition by Curcuma longarhizome extracts.

50

100

200

Extracts	Concentration (µg/ml)	% inhibition
	250	12.45 ± 2.65^{a}
Aqueous	500	24.45 ± 2.73^{b}
-	1000	$42.19 \pm 3.43^{\circ}$
	250	80.76 ± 3.78^{e}
Hydroethanolic	500	88.21 ± 3.92^{f}
	1000	$94.12\pm2.84^{\rm g}$
	250	25.75 ± 2.18^{b}
Ethanolic	500	$44.33 \pm 2.97^{\circ}$
	1000	62.52 ± 3.71^{d}

Values (n = 3) with different superscript letters are significantly different (p < 0.05) according to Waller-Duncan post hoc test.

TABLE 5: Correlation between total phenolic content (TPC), flavonoid content (FC), DPPH free radical scavenging (DPPH), ferric reducing antioxidant power (FRAP), and inhibition of alpha-amylase (IAA) of *Curcuma longa* rhizome powder extracts.

Parameters	TPC	FC	DPPH	FRAP	IAA
ТРС	1				
FC	0.976	1			
DPPH	1.000	0.969	1		
FRAP	1.000	0.971	1.000	1	
IAA	1.000	0.969	1.000	1.000	1

Correlation differs significantly at p < 0.01.

serum and liver levels of the markers of oxidative stress are presented in Table 9. It can be observed that serum and liver levels of malondialdehyde (MDA) and nitric oxide (NO) were significantly (p < 0.05) increased in high-fructose high-fat diet-fed rats (MDA: 67.54 ± 2.48 nmol/ml and 142.54 ± 6.15 nmol/mg and NO: 27.83 ± 1.44 nmol/ml and 83.92 ± 3.77 nmol/mg, respectively) compared to the base group rats (MDA: 36.02 ± 1.90 nmol/ml and 83.28 ± 5.30 nmol/mg and NO: 16.94 ± 2.16 nmol/ml and 25.81 ± 4.69 nmol/mg, respectively). *Curcuma longa* rhizome powder supplementation at 10%

significantly decreased (p < 0.05) the MDA and NO levels in the serum and liver in high-fructose high-fat diet-fed rats (MDA: $39.75 \pm 1.02 \text{ nmol/ml}$ and $92.71 \pm 2.98 \text{ nmol/mg}$ and NO: 19.36 ± 1.28 nmol/ml and 37.22 ± 4.34 nmol/mg, respectively) compared to the control group rats (MDA: 67.54 ± 2.48 nmol/ml and 142.54 ± 6.15 nmol/mg and NO: 27.83 ± 1.44 nmol/ml and 83.92 ± 3.77 nmol/mg, respectively). Nevertheless, catalase (CAT) activities of serum and liver were significantly decreased (p < 0.05) in high-fructose high-fat diet-fed rats $(4.12 \pm 0.56 \text{ U})$ min/ml and 6.54 ± 0.44 U/min/mg, respectively) compared to the base group rats $(14.59 \pm 1.43 \text{ U/min/ml} \text{ and } 17.10 \pm 1.62 \text{ U/}$ min/mg, respectively). The consumption of high-fructose highfat diet supplemented with Curcuma longa rhizome powder at 10% significantly increased (p < 0.05) the serum and liver CAT activities $(11.58 \pm 0.83 \text{ U/min/ml} \text{ and } 11.51 \pm 1.04 \text{ U/min/mg},$ respectively) compared to the control group rats $(4.12 \pm 0.56 \text{ U}/$ min/ml and 6.54 ± 0.44 U/min/mg, respectively).

3.9. Effect of Curcuma longa Rhizome Powder Supplementation on Body Weight and Food Intake. Table 10 lays out the body weight of rats during the treatment period. It appears that high-fructose high-fat diet-fed rats showed a significantly increased (p < 0.05)body weight gain $(316.75 \pm 2.34 \text{ g})$ compared to the base group rats $(270 \pm 6.37 \text{ g})$ after induction of metabolic disorders. Curcuma longa rhizome powder supplementation at 10% in high-fructose high-fat diet-fed rats significantly decreased (p < 0.05) the final body weight $(215.25 \pm 9.42 \text{ g})$ compared to the control group $(328.5 \pm 5 \text{ g})$. The weekly food intake of each rat and the averages are presented in Table 11. It shows that food intake was significantly (p < 0.05) decreased in high-fructose high-fat diet-fed rats $(31.14 \pm 0.78 \text{ g/day})$ compared to the base group rats $(38.21 \pm 0.74 \text{ g/day})$. Curcuma longa rhizome powder supplementation at 10% reduced significantly (p < 0.05) the food intake of highfructose high-fat diet-fed rats $(21.96 \pm 0.35 \text{ g/day})$ compared to the control group $(31.14 \pm 0.78 \text{ g/day})$ after supplementation.

Ethanolic

7

			Lee index			
Periods	Weeks	Group 1	Group 2	Group 3	Group 4	Group 5
	0	251.56 ± 1.57^{a}	250.88 ± 5.14^{a}	245.90 ± 3.78^{a}	253.43 ± 3.87^{a}	246.98 ± 5.37^{a}
In du ation	4	$263.23 \pm 3.53^{\rm b}$	269.74 ± 4.57^{b}	282.17 ± 4.55^{b}	$274.78 \pm 4.30^{ m b}$	$278.75 \pm 4.40^{ m b}$
Induction	8	$279.25 \pm 3.98^{\circ}$	$305.03 \pm 6.08^{\circ}$	$301.60 \pm 2.78^{\circ}$	$307.34 \pm 5.22^{\circ}$	$302.26 \pm 6.52^{\circ}$
	12	$287.43 \pm 4.32^{\circ}$	322.22 ± 7.13^{d}	315.24 ± 5.00^{d}	325.32 ± 7.53^{d}	326.88 ± 3.08^{d}
	1	287.55 ± 6.48^{a}	331.27 ± 8.37^{a}	313.01 ± 4.96^{a}	315.19 ± 7.23^{a}	313.83 ± 1.99^{a}
Supplementation	2	288.77 ± 6.70^{a}	328.08 ± 7.28^{a}	310.55 ± 5.07^{a}	$299.74 \pm 5.95^{\circ}$	291.30 ± 1.71^{b}
	3	287.06 ± 2.56^{a}	332.43 ± 7.32^{a}	306.79 ± 5.27^{a}	282.39 ± 5.49^{b}	$276.72 \pm 4.16^{\circ}$
	4	288.26 ± 3.58^{a}	332.43 ± 6.94^{a}	304.68 ± 5.63^{a}	280.79 ± 5.83^{b}	262.45 ± 4.71^{d}

TABLE 6: Effect of Curcuma longa rhizome powder supplementation on the Lee index in the high-fructose high-fat diet-fed rats.

Values (n = 4) with different superscript letters in the same row are significantly different (p < 0.05) according to Waller-Duncan post hoc test. Group 1 (base group): received basal diet; Group 2 (control group): received high-fructose high-fat diet; Group 3: received high-fructose high-fat diet supplemented with 2.5% *Curcuma longa* rhizome powder; Group 4: received high-fructose high-fat diet supplemented with 5% *Curcuma longa* rhizome powder; Group 5: received high-fructose high-fat diet supplemented with 10% *Curcuma longa* rhizome powder.

TABLE 7: Effect of the Curcuma longa rhizome powder supplementation on glycaemia (mg/dl) in the high-fructose high-fat diet-fed rats.

			Glycaemia (mg/dl	l)		
Periods	Weeks	Group 1	Group 2	Group 3	Group 4	Group 5
	0	129.00 ± 4.86^{a}	125.75 ± 6.92^{a}	126.25 ± 4.66^{a}	127.25 ± 5.13^{a}	127.00 ± 6.21^{a}
Induction	4	132.25 ± 3.40^{a}	152.25 ± 5.56^{b}	157.50 ± 7.23^{b}	$149.75 \pm 4.99^{ m b}$	153.75 ± 7.71^{b}
Induction	8	136.75 ± 5.96^{a}	$189.25 \pm 6.39^{\circ}$	$186.25 \pm 8.70^{\circ}$	$182.66 \pm 8.88^{\circ}$	$186.75 \pm 7.14^{\circ}$
	12	$134.50 \pm 3.10^{\rm a}$	$192.50 \pm 3.16^{\circ}$	$187.00 \pm 3.16^{\circ}$	$185.25 \pm 5.12^{\circ}$	$189.25 \pm 5.98^{\circ}$
	1	133.25 ± 2.75^{a}	185.50 ± 4.43^{a}	177.00 ± 4.96^{a}	170.00 ± 3.55^{a}	165.50 ± 3.10^{a}
Supplementation	2	135.50 ± 2.38^{a}	183.50 ± 4.43^{a}	168.25 ± 4.34^{b}	154.75 ± 2.98^{b}	146.25 ± 2.21^{b}
	3	132.50 ± 2.64^{a}	186.75 ± 3.36^{a}	160.75 ± 2.50^{b}	$141.25 \pm 2.21^{\circ}$	$128.00 \pm 3.91^{\circ}$
	4	136.00 ± 4.73^{a}	186.00 ± 6.94^{a}	$165.00 \pm 3.10^{\rm b}$	130.50 ± 3.69^{d}	$120.50 \pm 4.91^{\circ}$

Values (n = 4) with different superscript letters in the same row are significantly different (p < 0.05) according to Waller-Duncan post hoc test. Group 1 (base group): received basal diet; Group 2 (control group): received high-fructose high-fat diet; Group 3: received high-fructose high-fat diet supplemented with 2.5% *Curcuma longa* rhizome powder; Group 4: received high-fructose high-fat diet supplemented with 5% *Curcuma longa* rhizome powder; Group 5: received high-fructose high-fat diet supplemented with 10% *Curcuma longa* rhizome powder.

TABLE 8: Effect of the *Curcuma longa* rhizome powder supplementation on the lipid profile (mg/dl) in the high-fructose high-fat diet-fed rats.

Lipid profile (mg/dl)						
Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	
Triglycerides	128.43 ± 1.11^{b}	$158.35 \pm 5.37^{\circ}$	139.75 ± 2.99^{d}	116.41 ± 2.65^{e}	109.94 ± 1.42^{a}	
Total cholesterol	124.40 ± 4.63^{a}	208.53 ± 1.53^{b}	$156.44 \pm 2.41^{\circ}$	119.94 ± 3.86^{a}	114.18 ± 2.20^{d}	
HDL-cholesterol	59.44 ± 1.70^{d}	$48.12 \pm 1.82^{\circ}$	63.58 ± 1.13^{b}	74.21 ± 1.04^{a}	72.63 ± 1.59^{a}	
LDL-cholesterol	$39.26\pm3.78^{\rm b}$	$128.73 \pm 3.64^{\circ}$	$64.91 \pm 1.58^{\rm a}$	20.44 ± 3.61^{d}	$17.55 \pm 1.59^{\rm d}$	

Values (n = 4) with different superscript letters in the same line are significantly different (p < 0.05) according to Waller-Duncan post hoc test. Group 1 (base group): received basal diet; Group 2 (control group): received high-fructose high-fat diet; Group 3: received high-fructose high-fat diet supplemented with 2.5% *Curcuma longa* rhizome powder; Group 4: received high-fructose high-fat diet supplemented with 5% *Curcuma longa* rhizome powder; Group 5: received high-fructose high-fat diet supplemented with 10% *Curcuma longa* rhizome powder.

3.10. Effect of Curcuma longa Rhizome Powder Supplementation on Liver Weight and Fat Pad Deposition. The liver weights of all the rats in each group are presented in Table 12. From this table, it can be observed that the liver weights were significantly increased (p < 0.05) in highfructose high-fat diet-fed groups (3.61 ± 0.01 g/100 g of body weight) compared to the base group (2.60 ± 0.03 g/ 100 g of body weight) of rats. Curcuma longa rhizome powder supplementation at 5% significantly decreased (p < 0.05) the liver weights of high-fructose high-fat dietfed rats (3.05 ± 0.21 g/100 g of body weight). Fat deposition in high-fructose high-fat diet-fed rats is also presented in Table 12. It appears that the wet weight of peritoneal and mesenteric fat deposits was significantly increased (p < 0.05) in high-fructose high-fat diet-fed groups (2.18 ± 0.02 and 2.96 ± 0.03 g/100 g of body weight, respectively), compared to the base group (1.46 ± 0.06 and 1.82 ± 0.10 g/100 g of body weight, respectively). However, *Curcuma longa* rhizome powder supplementation at 10% in high-fructose high-fat diet-fed rats significantly reduced (p < 0.05) deposition of peritoneal and mesenteric fat (1.35 ± 0.14 and 1.65 ± 0.02 g/100 g body weight, respectively), compared to the control group (2.18 ± 0.02 and 2.96 ± 0.03 g/100 g body weight, respectively).

	Serum and liver levels of oxidative stress markers							
	Parameters	Group 1	Group 2	Group 3	Group 4	Group 5		
	NO (nmol/ml)	16.94 ± 2.16^{a}	27.83 ± 1.44^{d}	$24.08 \pm 0.76^{\circ}$	20.78 ± 1.56^{b}	19.36 ± 1.28^{b}		
Serum	MDA (nmol/ml)	36.02 ± 1.90^{a}	67.54 ± 2.48^{d}	$55.86 \pm 2.38^{\circ}$	41.96 ± 2.93^{b}	39.75 ± 1.02^{b}		
	CAT (U/min/ml)	14.59 ± 1.43^{d}	4.12 ± 0.56^{a}	7.45 ± 1.23^{b}	$10.92 \pm 1.65^{\circ}$	$11.58 \pm 0.83^{\circ}$		
	NO (nmol/mg)	25.81 ± 4.69^{a}	83.92 ± 3.77^{d}	$66.89 \pm 2.98^{\circ}$	42.51 ± 5.23^{b}	37.22 ± 4.34^{b}		
Liver	MDA (nmol/mg)	83.28 ± 5.30^{a}	142.54 ± 6.15^{d}	$121.24 \pm 3.36^{\circ}$	$97.45 \pm 3.05^{ m b}$	92.71 ± 2.98^{b}		
	CAT (U/min/mg)	17.10 ± 1.62^{d}	$6.54\pm0.44^{\rm a}$	$8.26\pm0.37^{\rm b}$	$12.46 \pm 1.19^{\circ}$	$11.51 \pm 1.04^{\circ}$		

TABLE 9: Effect of the *Curcuma longa* rhizome powder supplementation on serum and liver levels of oxidative stress markers in high-fructose high-fat diet-fed rats.

Values (n = 4) with different superscript letters in the same line are significantly different (p < 0.05) according to Waller-Duncan post hoc test. Group 1 (base group): received basal diet; Group 2 (control group): received high-fructose high-fat diet; Group 3: received high-fructose high-fat diet supplemented with 2.5% *Curcuma longa* rhizome powder; Group 4: received high-fructose high-fat diet supplemented with 5% *Curcuma longa* rhizome powder; Group 5: received high-fructose high-fat diet supplemented with 10% *Curcuma longa* rhizome powder.

TABLE 10: Effect of the Curcuma longa rhizome powder supplementation on the body weight gain (g) in high-fructose high-fat diet-fed rats.

	Body weight gain (g)							
Weeks	Group 1	Group 2	Group 3	Group 4	Group 5			
0	270.00 ± 6.37^{a}	316.75 ± 2.34^{a}	320.50 ± 8.50^{a}	318.75 ± 3.40^{a}	317.00 ± 6.98^{a}			
1	272.50 ± 5.12^{a}	319.50 ± 4.65^{a}	301.75 ± 9.81^{b}	280.25 ± 5.12^{b}	272.00 ± 6.99^{b}			
2	275.75 ± 4.90^{a}	322.00 ± 2.16^{ab}	$287.75 \pm 6.89^{\circ}$	$241.25 \pm 4.57^{\circ}$	$230.25 \pm 16.99^{\circ}$			
3	277.50 ± 7.93^{a}	325.75 ± 2.98^{b}	277.75 ± 6.23^{cd}	232.25 ± 3.86^{d}	$230.25 \pm 11.95^{\circ}$			
4	278.50 ± 4.93^{a}	328.50 ± 5.00^{b}	271.75 ± 5.05^{d}	228.00 ± 6.44^{d}	$215.25 \pm 9.42^{\circ}$			

Values (n = 4) with different superscript letters in the same row are significantly different (p < 0.05) according to Waller-Duncan post hoc test. Group 1 (base group): received basal diet; Group 2 (control group): received high-fructose high-fat diet; Group 3: received high-fructose high-fat diet supplemented with 2.5% *Curcuma longa* rhizome powder; Group 4: received high-fructose high-fat diet supplemented with 5% *Curcuma longa* rhizome powder; Group 5: received high-fructose high-fat diet supplemented with 10% *Curcuma longa* rhizome powder.

TABLE 11: Effect of Curcuma long	a rhizome powder su	upplementation on the fo	ood intake (g/day) i	in high-fructose high-fat diet-fed rats.

Food intake (g/day)								
Weeks	Group 1	Group 2	Group 3	Group 4	Group 5			
0	33.50 ± 1.09^{a}	34.50 ± 1.29^{a}	33.75 ± 1.25^{a}	34.25 ± 1.70^{a}	34.25 ± 0.95^{a}			
1	32.78 ± 0.99^{a}	34.07 ± 1.41^{a}	32.64 ± 1.02^{a}	33.96 ± 1.03^{a}	25.42 ± 1.13^{b}			
2	34.78 ± 0.66^{a}	30.78 ± 0.64^{b}	26.32 ± 0.55^{b}	23.60 ± 0.41^{b}	$19.60 \pm 1.64^{\circ}$			
3	38.21 ± 0.74^{a}	32.35 ± 0.84^{b}	27.35 ± 0.96^{b}	24.67 ± 0.75^{b}	21.53 ± 0.74^{d}			
4	38.42 ± 0.42^{a}	$31.14\pm0.78^{\rm b}$	27.42 ± 0.58^{b}	24.32 ± 0.37^{b}	21.96 ± 0.35^{d}			

Values (n = 4) with different superscript letters in the same row are significantly different (p < 0.05) according to Waller-Duncan post hoc test. Group 1 (base group): received basal diet; Group 2 (control group): received high-fructose high-fat diet; Group 3: received high-fructose high-fat diet supplemented with 2.5% *Curcuma longa* rhizome powder; Group 4: received high-fructose high-fat diet supplemented with 5% *Curcuma longa* rhizome powder; Group 5: received high-fructose high-fat diet supplemented with 10% *Curcuma longa* rhizome powder.

TABLE 12: Effect of *Curcuma longa* rhizome powder supplementation on liver wet weight and peritoneal and mesenteric fat wet weight (g/ 100 g of body weight) of high-fructose high-fat diet-fed rats.

Weight (g/100 g of body weight)									
Parameters	Group 1	Group 2	Group 3	Group 4	Group 5				
Liver weight	2.60 ± 0.03^{a}	3.61 ± 0.01^{b}	3.60 ± 0.02^{b}	$3.05 \pm 0.21^{\circ}$	$3.10 \pm 0.06^{\circ}$				
Peritoneal fat	1.46 ± 0.06^{a}	2.18 ± 0.02^{b}	1.91 ± 0.04^{c}	1.60 ± 0.02^{a}	1.35 ± 0.14^{a}				
Mesenteric fat	$1.82\pm0.10^{\rm a}$	$2.96\pm0.03^{\rm b}$	$3.01\pm0.19^{\rm b}$	2.42 ± 0.17^{c}	$1.65\pm0.02^{\rm a}$				

Values (n = 4) with different superscript letters in the same line are significantly different (p < 0.05) according to Waller-Duncan post hoc test. Group 1 (base group): received basal diet; Group 2 (control group): received high-fructose high-fat diet; Group 3: received high-fructose high-fat diet supplemented with 2.5% *Curcuma longa* rhizome powder; Group 4: received high-fructose high-fat diet supplemented with 5% *Curcuma longa* rhizome powder; Group 5: received high-fructose high-fat diet supplemented with 10% *Curcuma longa* rhizome powder.

3.11. Effect of Curcuma longa Rhizome Powder Supplementation on Hepatic Enzymes. Changes in the functions of hepatic enzymes are shown in Figure 1. Serum AST activity was significantly increased (p < 0.05) (84.21 ± 3.66 U/L) in high-fructose high-fat diet-fed rats compared to the base group (53.81 ± 1.12 U/L). Food supplementation with *Curcuma longa*

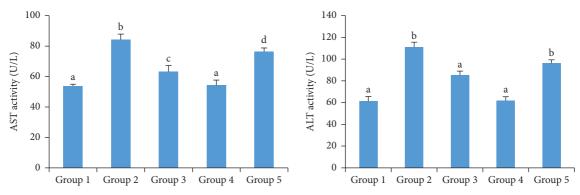


FIGURE 1: Effect of *Curcuma longa* rhizome powder supplementation on AST and ALT activities of high-fructose high-fat diet-fed rats. Values (n = 4) with different letters are significantly different (p < 0.05) according to Waller-Duncan post hoc test. Group 1 (base group): received basal diet; Group 2 (control group): received high-fructose high-fat diet; Group 3: received high-fructose high-fat diet supplemented with 2.5% *Curcuma longa* rhizome powder; Group 4: received high-fructose high-fat diet supplemented with 5% *Curcuma longa* rhizome powder; Group 5: received high-fructose high-fat diet supplemented with 10% *Curcuma longa* rhizome powder.

rhizome powder at 5% after four weeks significantly (p < 0.05) reduced the AST activity (54.25 ± 3.42 U/L), compared to the control group (84.21 ± 3.66 U/L). Serum ALT activities were also significantly increased (p < 0.05) in high-fructose high-fat diet-fed rats (111.12 ± 4.46 U/L) compared to the base group (61.46 ± 4.12 U/L). Food supplementation with *Curcuma longa* rhizome powder at 5% also reduced significantly (p < 0.05) the activities of these liver enzymes (61.90 ± 3.52 U/L) compared to the control group (111.12 ± 4.46 U/L).

3.12. Effect of Curcuma longa Rhizome Powder Supplementation on Haematological Parameters. Results of haematological parameters are depicted in Table 13. It appears that induction of metabolic disorder leads to a significant decrease (p < 0.05) in white blood cells (WBC), red blood cells (RBC), haemoglobin (HGB), haematocrit (HCT), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and platelet (PLT) levels $(1.90 \pm 0.16 \ 10^9/L, \ 4.40 \pm 0.35 \ 10^{12}/L,$ 10.10 ± 1.29 g/dl, $30.32 \pm 1.91\%$, 27.72 ± 1.20 g/dl, 57.50 ± 2.03 fl, 17.25 ± 0.54 pg, and $690.2 \pm 21.6 \ 10^9$ /L, respectively) compared to the base group $(2.72 \pm 0.22 \ 10^9/L, \ 6.59 \pm 0.36 \ 10^{12}/L,$ 13.40 ± 1.09 g/dl, $37.55 \pm 2.40\%$, 34.10 ± 1.75 g/dl, 62.57 ± 0.78 fl, 21.37 ± 0.97 pg, and $768.2 \pm 23.1 \ 10^{9}$ /L, respectively). Feeding on Curcuma longa rhizome powder supplementation at 5% significantly (p < 0.05) increased their level ($2.75 \pm 0.26 \ 10^9/L$) $8.36 \pm 0.29 \ 10^{12}$ /L, $16.67 \pm 1.06 \text{ g/dl}$, $56.20 \pm 1.47\%$, 37.27 ± 1.75 g/dl, 69.95 ± 2.01 fl, 24.05 ± 1.32 pg, and $935.5 \pm 43.9 \ 10^9$ /L, respectively), compared to the control group rats $(1.90 \pm 0.16 \, 10^9/L)$, $4.40 \pm 0.35 \ 10^{12}$ /L, 10.10 ± 1.29 g/dl, 30.32 ± 1.91 %, 27.72 ± 1.91 % 1.20 g/dl, $57.50 \pm 2.03 \text{ fl}$, $17.25 \pm 0.54 \text{ pg}$, and $690.2 \pm 21.6 \ 10^9/\text{L}$, respectively). A significant (p < 0.05) reduction was observed in the lymphocyte (LYMP) concentration of high-fructose high-fat diet-fed rats $(2.07 \pm 0.12 \ 10^9/L)$ compared to the base group $(2.86 \pm 0.17 \ 10^9/L)$. Feeding on the Curcuma longa rhizome powder supplementation leads to no significant increase (p > 0.05) $(2.15 \pm 0.12 \, 10^9/L)$ compared to the control group rats $(2.07 \pm 0.12 \ 10^9/L)$. A significant (p < 0.05) increase in the level of monocytes (MCT) was observed in the untreated metabolic disorders rats $(0.37 \pm 0.00 \ 10^9/L)$ compared to the base group

 $(0.26 \pm 0.05 \ 10^9/L)$. A significant (p < 0.05) reduction level was observed in the *Curcuma longa* rhizome powder supplementation 5% diet-fed rats $(0.23 \pm 0.07 \ 10^9/L)$ compared to the control group $(0.37 \pm 0.00 \ 10^9/L)$.

4. Discussion

Plants' natural products represent the safest, effective, and alternative source of chemical drugs. The latter possess side effects; hence, researchers are turning to the use of plants to solve health problems. In the current study, the results of the phytochemical analysis showed that the TPC in the hydroethanolic extract was higher than that of Najah et al. [39] $(51.46 \pm 0.46 \text{ mg GAE/g})$ on methanol/chloroform extracts of Curcuma longa rhizome powder. In contrast, the TPC obtained in this study was significantly less than those reported by Choi [40] in ethyl acetate and chloroform extracts of Curcuma longa rhizome from Korea with respective values of 228.7 ± 2.3 and 140.7 ± 10.6 mg GAE/g extract. According to Shan et al. [41], the differences in the level of TPC and FC may be due to the type of solvent used for extraction, time of harvesting samples, determination methods, and the differences in the geographical conditions from one country to another such as climate, temperature, fertility, and disease.

Regarding antioxidant activity results, the higher TPC in hydroethanolic extract (about 1.30-fold that of ethanolic extract and 1.57-fold that of aqueous extract) might explain its higher DPPH radical scavenging activity. These results are in accordance with those of Najah et al. [39] and Sabir et al. [42] which showed that DPPH scavenging activity of methanol/chloroform and ethanolic extract of Curcuma longa rhizome increased with the TPC. Also, the highest FRAP activity observed with the hydroethanolic extract may be associated with the high amount of hydroxyl groups thanks to phenolic compounds such as flavonoids that can play the role of electron donors, hereby reducing Fe^{3+} to Fe^{2+} [43]. An increase in the quantity of Fe^{2+} results in the rise of the reducing power of the extract. As such, antioxidants are considered as reducers and inactivators of oxidants [44].

Haematological parameters								
Parameters	Group 1	Group 2	Group 3	Group 4	Group 5			
HCT (%)	37.55 ± 2.40^{a}	30.32 ± 1.91^{e}	47.20 ± 1.49^{b}	56.20 ± 1.47^{d}	46.87 ± 2.79^{b}			
RBC (10 ¹² /L)	6.59 ± 0.36^{a}	4.40 ± 0.35^{e}	$7.67 \pm 0,50^{ m b}$	8.36 ± 0.29^{bc}	$7.79 \pm 0.30^{ m b}$			
HGB (g/dl)	13.40 ± 1.09^{a}	10.10 ± 1.29^{d}	14.40 ± 0.52^{a}	$16.67 \pm 1.06^{\circ}$	13.37 ± 1.09^{a}			
MCHC (g/dl)	$34.10 \pm 1.75^{\circ}$	27.72 ± 1.20^{a}	$33.15 \pm 2.47^{\circ}$	37.27 ± 1.75^{b}	26.82 ± 1.64^{a}			
MCV (fl)	62.57 ± 0.78^{b}	57.50 ± 2.03^{a}	62.42 ± 1.07^{b}	$69.95 \pm 2.01^{\circ}$	56.70 ± 2.11^{a}			
MCH (pg)	21.37 ± 0.97^{b}	17.25 ± 0.54^{a}	19.52 ± 0.83^{a}	$24.05 \pm 1.32^{\circ}$	18.20 ± 0.94^{a}			
WBC (10 ⁹ /L)	2.72 ± 0.22^{b}	1.90 ± 0.16^{a}	2.60 ± 0.29^{b}	2.75 ± 0.26^{b}	2.77 ± 0.29^{b}			
E (10 ⁹ /L)	0.30 ± 0.02^{b}	0.25 ± 0.07^{b}	$0.10 \pm 0.00^{\mathrm{a}}$	$0.35 \pm 0.07^{\rm bc}$	$0.10 \pm 0.00^{\mathrm{a}}$			
MCT (10 ⁹ /L)	0.26 ± 0.05^{a}	$0.37 \pm 0.00^{\mathrm{b}}$	0.16 ± 0.05^{a}	0.23 ± 0.07^{a}	0.2 ± 0.01^{a}			
LYMP (10 ⁹ /L)	2.86 ± 0.17^{b}	2.07 ± 0.12^{a}	2.12 ± 0.17^{a}	2.15 ± 0.12^{a}	1.97 ± 0.17^{a}			
PLT (10 ⁹ /L)	768.2 ± 23.1^{b}	690.2 ± 21.6^{a}	723.7 ± 23.9^{a}	$935.5 \pm 43.9^{\circ}$	$781.5 \pm 16.0^{ m b}$			

TABLE 13: Effect of *Curcuma longa* rhizome powder supplementation on haematological parameters.

Values (n = 4) with different superscript letters in the same line are significantly different (p < 0.05) according to Waller-Duncan post hoc test. Group 1 (base group): received basal diet; Group 2 (control group): received high-fructose high-fat diet; Group 3: received high-fructose high-fat diet supplemented with 2.5% *Curcuma longa* rhizome powder; Group 4: received high-fructose high-fat diet supplemented with 5% *Curcuma longa* rhizome powder; Group 5: received high-fructose high-fat diet supplemented with 5% *Curcuma longa* rhizome powder; Group 5: received high-fructose high-fat diet supplemented with 10% *Curcuma longa* rhizome powder. HCT = haematocrit; RBC = red blood cells; HGB = haemoglobin; MCH = mean corpuscular haemoglobin concentration; MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin; WBC = white blood cells; E = erythrocytes; MCT = monocytes; LYMP = lymphocytes; PLT = platelets.

The in vitro inhibition of alpha-amylase by the different extracts of Curcuma longa rhizome showed a dosedependent increase in the percentage of inhibition. The phytochemical compounds present in the extracts could account for the inhibition of the digestive enzymes that are needed for the production of energy [45]. These compounds have hydroxyl functions similar to the substrates of amylase, which can induce competitive inhibition of the enzyme. Aqueous extract presented the lowest activity, which could be due to the low total phenolic and flavonoid contents, while hydroethanolic extract showed the best inhibitory activity of alpha-amylase because of the high TPC and FC. These results are consistent with those of Manikandan et al. [31] and Henda et al. [46] that demonstrated that extracts from Psidium guajava and Juniperus phoenicea leaves, respectively, rich in phytochemical compounds presented inhibitory activities of alpha-amylase.

Looking at the significant positive correlation recorded between the phytochemical compounds' contents and the antioxidant properties of the different *Curcuma longa* rhizome powder extracts, there is a relation between the TPC, FC, DPPH, and FRAP because the extract that shows a high antioxidant activity, taking into consideration the DPPH and FRAP, also has the highest TPC and FC. The results showing the positive and significant correlation between TPC, DPPH, and FRAP are in line with those of Choi [40] who showed a significant positive correlation between these parameters with methanolic extract of *Curcuma longa* rhizome.

The *in vitro* antioxidant and amylase inhibitory activities of *Curcuma longa* rhizome powder were used to assess its effect on metabolic parameters in rat-induced metabolic disorders. The consumption of a high-fat and high energy diet and sedentary lifestyles are recognised as paramount causes of the development of metabolic disorders [47, 48]. As shown in this study, rats that ate a high-fructose high-fat diet developed obesity by increasing their Lee index. Lee index is a major parameter for assessing the effect of a highfructose high-fat diet on the development of obesity and for monitoring its treatment. When the Lee index of an animal is greater or equal to 300, it is obese [34]. The development of obesity was due to the excess of fat contained in the hyperlipidic diet, leading to an imbalance between food energy intake and the energy expenditure necessary for daily activities. Moreover, the consumption of a high-fructose high-fat diet supplemented with Curcuma longa rhizome powder significantly decreased Lee index of the obese rats; this may be due to the presence of phytochemical compounds in the powder that inhibited digestive enzymes and decreased the energy intake of animals [49]. The glycaemia test performed during the study also showed an increase in blood glucose level, and this may be due to the deposition of fat at the level of pancreas tissues that block the insulin activity and lead to a higher blood glucose level in rats fed with a high-fructose high-fat diet. These results were similar to those of Shoumen et al. [47] and Akiyama et al. [50], who showed that rats fed on a high-fructose high-fat diet developed hyperglycemia. Moreover, Curcuma longa rhizome powder supplementation decreased the glycaemia of the high-fructose high-fat diet-fed rats. These results could be due to the presence of phytochemical compounds in the Curcuma longa rhizome powder which inhibited the digestion of carbohydrates and as such reduced the absorption of glucose and postprandial blood glucose level [51].

Regarding the lipid profile results, the consumption of a high-fructose high-fat diet induced metabolic disorders with an increase in serum total cholesterol, LDLc, and triglycerides levels compared to the base group, while HDLc remained low. This could be justified by the fact that the fat (beef suet) used for the preparation of the high-fat diet has caused hypercholesterolemia with the deposit of cholesterol plaques in the vascular walls in rats suffering from metabolic disorders. The more severe the metabolic disorder is, the more significant the HDLc decreases [52]. This decrease in good cholesterol (HDLc) and increase in bad cholesterol (LDLc) is due to high blood glucose levels, which is a sign of type 2 diabetes [53]. Based on the current investigation, Curcuma longa rhizome powder supplementation reduced the triglycerides, total-cholesterol, and LDL-cholesterol and increased the HDL-cholesterol in high-fructose high-fat diet-fed rats. The decrease in cholesterol rate in rats that consumed Curcuma longa rhizome powder could be due to the absorption of phytochemical compounds which inhibited the activity of hydroxymethylglutaryl-CoA reductase. This enzyme permits endogenous synthesis of cholesterol by transforming acetyl coenzyme-A in mevalonic acid, an intermediate in the synthesis chain of endogenous cholesterol [54]. This result could also be due to the presence of polyunsaturated fatty acids in Curcuma longa rhizome powder since the works of Munshi et al. [55] have shown that oil extracted from Curcuma longa rhizome has important content of linoleic and linolenic acids. Adipogenesis, known as cell formation, is a differentiation process which converts undifferentiated preadipocytes into fully differentiated adipocytes that store energy in the form of fat and render subjects obese [56]. Adipose tissue is a dynamic organ whose mass changes throughout life in response to the animal's metabolic needs and therefore plays an important role in energy balance. Obesity-associated genes such as leptin, adiponectin, and fatty acid synthase regulate adipogenesis and lipid metabolism at different stages of adipocyte differentiation [57]. It has been reported that excess fat is positively correlated with leptin concentration in rodents and humans [58]. Leptin is a protein secreted by adipocytes that plays a crucial role in the regulation of body weight by controlling the size of adipose tissue. It regulates food intake by binding to receptors in the central nervous system and modulating the activity of neurons present in the brain's appetite control centers [59]. In our study, the phytochemical compounds present in the powder could lower leptin [60] expression in animals fed with a highfructose high-fat diet supplemented with Curcuma longa rhizome powder, resulting in a drop in food intake (reduced appetite), which in turn could lead to a reduction in serum triglyceride and cholesterol levels, which have a direct impact on the amount of fat stored in adipocytes.

Adiponectin, acting as an antagonist of adipogenesis, plays an effective role in regulating lipid and glucose metabolism in insulin-sensitive organs in animals and humans [61]. Low circulating adiponectin levels have been demonstrated in genetic and diet-induced obesity models [62]. It has been reported that upregulation of adiponectin levels is inversely correlated with fat mass and insulin resistance [63]. Therefore, in our study, the decrease in blood glucose, serum lipid concentrations, and body weight in rats receiving a high-fructose high-fat diet supplemented with Curcuma longa rhizome powder may be due to the upregulation of adiponectin levels by the phytochemicals present in the powder. The antiobesity activity observed in rats fed a high-fructose high-fat diet supplemented with Curcuma longa rhizome powder could be due to the property of phytochemical compounds that have the capacity to modify energy expenditure by increasing the expression of lipolytic proteins or the inhibition of lipogenesis by reducing the expression of fatty acid synthase. Similar reports of body

weight reduction have been reported previously with extracts of some plant species [60, 64-66]. The consumption of high-fructose high-fat diet in most experimental models to induce metabolic disorder also caused oxidative stress [47]. As shown in the present experiment, the levels of lipid peroxidation products (MDA and NO) were increased in high-fructose high-fat diet-fed rats. This increase in oxidative stress molecules might be associated with the decrease in CAT activity recorded in the present work. These results were congruous with previous studies revealing that tissue antioxidant defenses may be compromised in high-fructose high-fat diet-fed animals [67, 68]. Curcuma longa rhizome powder supplement prevented oxidative stress and restored the tissue antioxidants in high-fructose high-fat diet-fed rats. This result may be explained by the presence of phytochemical compounds that positively modulate free radical metabolism by increasing the activities of antioxidant enzymes and decreasing the concentration of lipid peroxidation products (MDA and NO) [69].

Experimentally, the use of a high-fructose high-fat diet permits us to get closer to the new nutritional habits of industrialised countries. The results of the present work showed that the consumption of a high-fructose high-fat diet significantly increased the body weight gain of the animals. This increase in body weight gain may be due to the presence of fats in the food that is likely to be accumulated in adipose tissue, leading to the development of obesity [68, 70]. Curcuma longa rhizome powder supplementation significantly decreased the body weight gain of the high-fructose high-fat diet-fed rats. This result can be justified by the presence of phytochemical compounds in the powder that inhibited digestive enzymes and reduced the absorption of nutrients [45]. The results of liver weight and fat deposition showed an increase in high-fructose high-fat diet-fed rats. However, Curcuma longa rhizome powder supplemented rats exhibited significantly reduced liver weight and deposition of peritoneal and mesenteric fats. This result may be due to the presence of phytochemical compounds in the Curcuma longa rhizome powder that inhibited the digestive enzymes in a consequence of the utilisation of fats stored in adipose tissue for the production of energy necessary for the functioning of the organism. This leads to the reduction of peritoneal and mesenteric fats as well as the weight of the liver. These results are in line with those of Manikandan et al. [31]. The hepatic damage was evaluated by measuring the activities of the liver enzyme markers. Serum ALT and AST increase when hepatic damage occurs and the activities of these enzymes were measured in all groups. The present study showed that the serum AST and ALT levels increased in high-fructose high-fat diet-fed rats compared to the base group. Hepatocyte damage caused by the consumption of a high-fructose high-fat diet is evident in most experimental models and patients with clinical conditions [50]. These enzymes are considered markers of hepatic dysfunction. Generally, hepatocyte damage causes these enzymes to be transported in the serum [71]. Induction of metabolic disorder results in the beginning of oxidative stress in several tissues, including the liver. This leads to the peroxidation of membrane lipids, altering hepatic lipid profile, which

consequently cause cellular damage and membrane rupture, with the release of AST and ALT into the bloodstream [72, 73].

The haematological parameters' results showed that the induction of metabolic disorder leads to a significant decrease in white blood cells (WBC), red blood cells (RBC), haemoglobin (HGB), haematocrit (HCT), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and platelet (PLT) level. Feeding on Curcuma longa rhizome powder supplementation significantly increased their level. Nutrients in food play vital roles in the normal function of the body. Inclusions of active secondary metabolites in human nutrition have been demonstrated to have health benefits and reduce the risk of chronic diseases such as cancer, obesity, diabetes, and anaemia [67, 70]. Reduction in these parameters could be due to the coexistence of metabolic disorder and anaemia in the same animal. HCT stands for erythrocyte volume fraction of the percentage of red blood cells. RBC are involved in the transport of oxygen and absorb nutrients [74]; thus a high HCT value shows better transportation of oxygen. Elsewhere, WBC help the organism's defense through phagocytosis, transportation, and distribution of antibodies, leading to immunity. Thus, individuals with low levels of WBC are exposed to a high risk of infection [75]. The platelet count is one of the most important screen tests of platelet functions. A decrease in the circulating platelet of less than 15% of the normal value will cause bleeding [76]. The reduction observed in the platelet count in the blood of high-fructose high-fat diet-fed rats compared to the base group may suggest a possible effect on blood clotting in rats suffering from metabolic disorders. In other findings, haemoglobin levels also decreased in rats suffering from metabolic disorders, probably due to the presence of anaemia. Erythrocyte indices do not define the cause of anaemia but rather may be useful during the diagnostic workup. These results are supported by those of Monteomo et al. [77] and Ochuko et al. [78] which show that rats have low haemoglobin concentration and haematocrit after consuming a high-fat diet and have similarities with iron deficiency anaemia. Changes in the haematology parameters in metabolic disorders were dominated by hypochromic anaemia corresponding to a decrease in erythrocyte, haemoglobin, haematocrit, reticulocyte contents (MCH and MCHC), and MCV values. The increase in the haematology parameters after food supplementation with Curcuma longa rhizome powder may be due to the presence of phytochemical compounds in this rhizome, but the mechanism still remains unknown.

5. Conclusion

This study reveals that the consumption of a high-fructose high-fat diet alters the normal biochemical parameters and leads to the development of metabolic disorders (obesity, hyperglycaemia, and dyslipidaemia) and oxidative stress. Food supplementation with *Curcuma longa* rhizome powder, an excellent source of phytochemical compounds, improved these metabolic parameters and oxidative stress markers by a significant reduction of Lee index, glycaemia, triglyceride, total-cholesterol, LDLc, MDA, and NO levels. This supplementation also upgraded haematological parameters (WBC, RBC, HGB, HCT, MCHC, MCV, MCH, and PLT) with the best hepatoprotective effects at 5% supplementation. Further research is needed to confirm these beneficial effects on a human subject through a clinical trial.

Data Availability

The data that support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

FZN and PMK conceived and designed the experiment and contributed reagents and materials. RKT collected the samples and performed the experiments with DSDB. MBDS and STT performed statistical analyses of data and worked with RKT in writing the manuscript. MEFK reviewed the manuscript with BN. All the authors have read and approved the final manuscript.

Acknowledgments

We acknowledge Dr. Ngouenam Romial Joel, Mr. Tekou Amel Florian, Dr. Tchamani Piame Laverdure, and Dr. Fotso Techeu Ulrich Daquain for their support during the experiments.

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