

Bioactive Components and Antioxidant Properties of *Acacia nilotica* Seeds: Exploring Their Potential as a Cholesterol-Lowering Agent in Rats

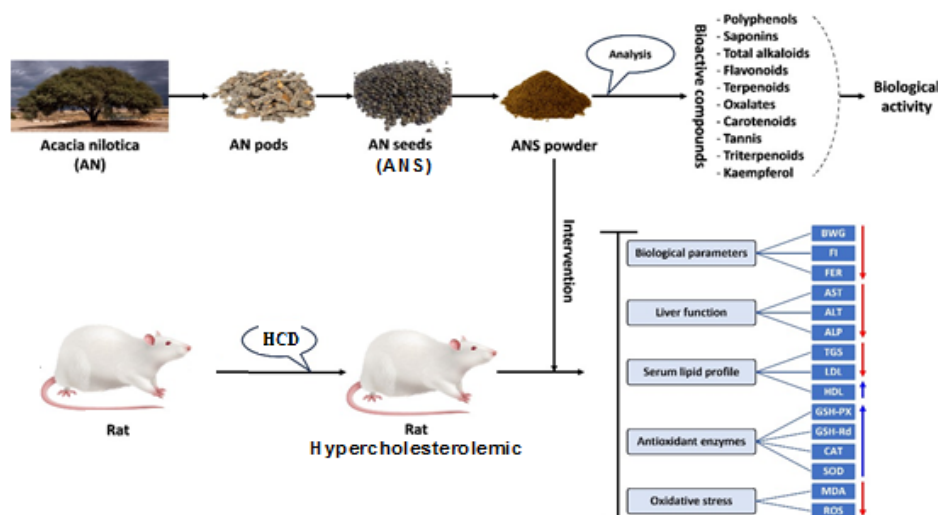
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Abstract This study examined the chemical composition, bioactive compounds, antioxidant activity of *Acacia nilotica* seed powder (ANS), and its effects on hypercholesterolemic rats over four weeks. Thirty-six rats were acclimated on a basal diet (BD) for two weeks, then divided into groups: a negative control on BD, a positive control fed a hypercholesterolemic diet (HCD) for 3 weeks, followed by BD, and three treatment groups receiving BD with 5%, 7%, and 9% ANS. Proximate analysis of ANS revealed 8.27% moisture, 9.43% crude fat, 27.13% protein, and 39.44% carbohydrates, indicating high nutritional value. Bioactive compounds included polysaccharides, polyphenols, saponins, alkaloids, flavonoids, and kaempferol, with low oxalates and tannins within safe limits. Antioxidant activity was 66.89%, comparable to standard antioxidants. ANS treatment improved metabolic parameters dose-dependently. Body weight gain (BWG), reduced by ~40% in untreated rats, improved by 10%, 20%, and 43% at 3, 6, and 9 g/100 g ANS, respectively. Feed intake and feed efficiency ratio similarly recovered. Liver enzymes AST, ALT, and ALP, elevated in the model by 39.8%, 61.8%, and 16.6%, decreased significantly with 9 g ANS by 22.1%, 95.5%, and 10.7%. Serum triglycerides and total cholesterol, increased by ~181% and 38%, declined dose-dependently with ANS, with TG dropping by ~260% and TC by ~22.7% at 9 g. HDL cholesterol rose by 25%, 37%, and 72%, while LDL and VLDL cholesterol fell by up to 44% and 41%. Glutathione levels (GSH and GSSG) improved, reversing 23.14% and 19.84% declines in controls. Erythrocyte antioxidant enzymes (GSH-Px, GSH-Rd, SOD, CAT) decreased by 25–38% in controls but increased by 23–45% with ANS. Oxidative stress markers ROS and MDA, elevated by 45% and 62.48%, were reduced dose-dependently, with MDA decreasing by 42.89% at the highest dose. These results demonstrate ANS's potent antioxidant and lipid-lowering effects in hypercholesterolemic rats.



Keywords: Hypercholesterolemia, liver functions, serum lipid profile, antioxidant enzymes, oxidative stress

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1. Introduction

Hypercholesterolemia refers to elevated levels of cholesterol in the blood. It is a type of "hyperlipidemia" (increased lipid levels in the blood) and "hyperlipoproteinemia" (increased lipoprotein levels in the blood). Cholesterol, a sterol, is one of the three main classes of lipids utilized by animal cells to build their membranes and is therefore produced by all animal cells. However, plant cells do not produce cholesterol. Cholesterol serves as a precursor for steroid hormones, bile acids, and vitamin D [1]. Since cholesterol is water-insoluble, it is carried through the blood plasma within protein particles known as lipoproteins. These lipoproteins are categorized by their density: very low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL) [2]. While all lipoproteins transport cholesterol, high levels of lipoproteins other than HDL (referred to as non-HDL cholesterol), particularly LDL cholesterol, are linked to an increased risk of atherosclerosis and coronary heart disease [3]. On the other hand, higher levels of HDL cholesterol are considered protective [4]. Elevated non-HDL cholesterol and LDL levels may result from various factors such as diet, obesity, genetic conditions (like LDL receptor mutations in familial hypercholesterolemia), or diseases like diabetes and hypothyroidism [1]. To reduce total blood cholesterol and LDL levels in adults, lowering dietary fat intake is recommended [5]. In cases of extremely high cholesterol (e.g., familial hypercholesterolemia), dietary changes alone may not be sufficient to achieve the desired reduction in LDL levels, and lipid-lowering medications that reduce cholesterol production or absorption are generally necessary. In severe cases, additional treatments, such as specialized LDL therapy or even surgery (especially for certain subtypes of familial hypercholesterolemia), may be required [6].

Plants still remain a major source for drug discovery in spite of the great development of synthetic molecules. Consequently, the uses of traditional plant extract in the treatment of various diseases have been flourished [7]. *Acacia nilotica*, also known as the gum arabic tree, is a versatile species from the *Fabaceae* family, native to tropical Africa, the Middle East, and the Indian subcontinent. This deciduous tree, thriving in dry and arid regions, is noted for its ability to adapt to various ecosystems, making it ecologically significant and economically valuable. Beyond its use in traditional medicine and agriculture, *Acacia nilotica* is particularly renowned for its gum, utilized in food, cosmetics, and pharmaceuticals [8]. The seeds of this tree have drawn increasing attention due to their substantial nutritional and therapeutic potential. From a botanical perspective, *Acacia nilotica* belongs to the *Acacia* genus, which includes over 1,000 species. It grows up to 20 meters tall and features light green bipinnate leaves and fragrant yellow flowers, arranged in cylindrical clusters. The tree produces a leguminous pod with hard, dark-colored, oval seeds that mature during the dry season, aiding their wide distribution via animal dispersal [9].

The seeds are particularly noteworthy for their rich macronutrient profile, including proteins, fats, and carbohydrates, which make them an important food source in regions facing food insecurity. They are encased in a hard shell, ensuring their long-term viability in harsh conditions. The high energy content of the seeds allows them to serve as a reliable source of calories, often consumed in processed forms such as flour or paste [10]. Recent research has highlighted the nutritional significance of these seeds, revealing protein content ranging from 22% to 30%, along with 5-8% fats, including essential fatty acids. These attributes make them a viable alternative to animal proteins in areas where these are scarce or costly [11]. Furthermore, the seeds contain approximately 50% carbohydrates, primarily in the form of starch, which provides slow-releasing energy, making it beneficial for blood sugar regulation. The fiber content, ranging from 7-10%, aids digestive health, and the seeds are rich in essential minerals such as calcium, potassium, and magnesium [9].

In addition to macronutrients, the chemical composition of *Acacia nilotica* seeds is characterized by bioactive compounds, including polyphenols, flavonoids, tannins, alkaloids, saponins, and glycosides. These compounds offer significant therapeutic potential due to their antioxidant, anti-inflammatory, and antimicrobial properties [8]. Among these, flavonoids have been particularly noted for their role in reducing oxidative stress, a key factor in aging and chronic diseases like cardiovascular diseases and cancer [12]. The seeds also contain compounds with potential in managing metabolic diseases such as hypertension and hypercholesterolemia. Studies have indicated that *Acacia nilotica* seed extracts can lower cholesterol by inhibiting lipid peroxidation and improving lipid metabolism, positioning it as a candidate for developing natural treatments for hypercholesterolemia and related disorders [13,14].

Beyond their nutritional value, *Acacia nilotica* seeds also possess bioactive compounds that have proven therapeutic benefits. The seeds' high fiber content helps regulate blood sugar and cholesterol levels, contributing to the management of diabetes and cardiovascular diseases [8]. Additionally, flavonoids and tannins in the seeds support immune function and possess anti-inflammatory properties, which can aid in treating conditions like arthritis and asthma [12]. Notably, *Acacia nilotica* seed extracts have been shown to have lipid-lowering effects, making them promising for managing hypercholesterolemia. Studies have demonstrated that these extracts significantly reduce total cholesterol, triglycerides, and LDL cholesterol levels while increasing HDL cholesterol, highlighting their potential in protecting against cardiovascular diseases [14].

Current investigations into *Acacia nilotica* seeds highlight their significant promise in enhancing human health, particularly in areas related to nutrition and disease prevention. Nonetheless, additional research is necessary to isolate and identify the specific bioactive compounds within the seeds and to gain a deeper understanding of how they function. As a result, the aim of this study was to analyze the chemical composition, bioactive compound content, and antioxidant properties of *Acacia nilotica* seed powder (ANS). Furthermore, the study explored the impact of a four-week treatment with *Acacia nilotica*

seeds on rats with induced hypercholesterolemia.

2. Materials and Methods

2.1. Materials

2.1.1. Acacia Nilotica Seeds

Dried and matured fruit pods used for seeds preparation were obtained from of *Acacia nilotica* trees spread at Santa Center Villages, Gharbia Governorate, Egypt after the grateful facilities provided to the authors. Taxonomic confirmation of *Acacia nilotica* pods were carried out by Agricultural Plant Department, Faculty of Agriculture, Menoufia University, Shebin El-Kom, Egypt.

2.1.2. Chemicals and Kits and Machines

Cholesterol and bile salts were purchased from ElGhohorya Company for Trading Drugs, Chemicals and Medical Suppliers, Cairo Egypt. Kit's assays for Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), malondialdehyde (MDA) were purchased from BIODIAGNOSTIC, Dokki, Giza, Egypt. Albumin (Alb), total protein (TP), triglycerides (TGs), cholesterol (Cho), high density lipoprotein (HDL) and low density lipoprotein (LDL) were determined using kits purchased from El-Nasr Pharmaceutical Chemicals Company, Cairo, Egypt. GSH and GSSG were assayed by the kits provided by MyBioSource, Inc., San Diego, CA, USA. Casein was obtained from Morgan Chemical Co., Cairo, Egypt. Vitamins and salts mixtures, organic solvents, buffers, and all other chemicals were obtained in analytical grade from El-Ghohorya Company for Trading Drugs, Chemicals, and Medical Instruments, Cairo, Egypt. Throughout this study absorbance for different assays were measured using UV-160A; Shimadzu Corporation, Kyoto, Japan.

2.2. Methods

2.2.1. Preparation of *Acacia nilotica* seeds Powder (ANS)

Seeds of *Acacia nilotica* were extracted from the fruits/pods manually using special sharp knives for this purpose. The collected seeds were manually sorted to exclude damaged and deformed ones, as well as foreign bodies. Seeds were dried in oven at 60°C for one hour and ground into a fine powder in high mixer speed (Moulinex Egypt, Al-Araby Co., Egypt). The material that passed through an 80 mesh sieve was retained for use.

2.2.2. Proximate Composition of *Acacia nilotica* Seeds (ANS)

Acacia nilotica seeds sample (ANS) were analyzed for proximate composition including moisture (using oven method at 105°C for 4 h), protein (T.N. $\times$ 6.25, using Kjeldahl method through oxidation, distillation, and titration, semiautomatic apparatus, Velp company, Italy), fat (soxhelt semiautomatic apparatus Velp company, Italy, petroleum ether solvent), ash (dry ashing method, muffle

furnace at 600°C up to material becoming ash) crude and dietary fibers content were determined using the official methods of analysis of the Association of Official Analytical Chemists (AOAC, (1995). Carbohydrates calculated by difference using the following formula: Carbohydrates (%) = 100 – (% protein + % fat + % Ash + % fiber).

2.2.3. Bioactive Compounds Extraction and Determination in *Acacia nilotica* Seeds (ANS)

Acacia nilotica seeds (ANS) was prepared/extracted for bioactive compounds determination according to the method of Gharib *et al.*, [15]. In brief, A 5 g of dried powder of *Acacia nilotica* seeds were extracted in a Soxhlet apparatus (Soxhlet Semiautomatic apparatus Velp Company, Italy) for 4-5 h (20 $\pm$ 4 min per cycle) using 80% hydro-ethanol (Ethanol:H₂O by 80 : 20%). Ethanol was evaporated in a rotary evaporator (Büchi R-210, Switzerland) to obtain the dried solvent extract and stored at 4 °C until use. The total yield of GLE was 2.64% (w/w) in terms of the *Acacia nilotica* seeds. The total phenolic content in ANS was quantified using the Folin-Ciocalteu reagent, as described by Singleton and Rossi, [16] and Wolfe *et al.*, [17], with results expressed as mg gallic acid equivalent.100 g⁻¹. The total carotenoid content in the 80% acetone extract was measured using the method outlined by Litchenthaler, [18] and expressed as mg catechin equivalent.100 g⁻¹. Total flavonoids were estimated using the colorimetric method of Zhisen *et al.*, [19], with results expressed as mg resveratrol equivalent,.100 g⁻¹. Polysaccharides were extracted and quantified following the procedure of Vazirian *et al.*, [20], using starch as the standard and the results were expressed as g starch equivalent. 100 g⁻¹. Total terpenoids were extracted and measured as per the method of Ghorai *et al.* [21], with linalool used as a standard and results presented as mg linalool equivalent. 100 g⁻¹. Total triterpenoids were assessed according to Schneider *et al.*, [22], using ursolic acid as the standard, with results expressed in mg ursolic acid equivalent.100 g⁻¹. Tannin content was determined using the method of Van-Burden and Robinson, [23], with catechine as the standard for constructing the calibration curve to estimate tannin levels as mg catechine equivalent. 100 g⁻¹. Total alkaloids were determined using the method of Zhao and Wang [24], with atropine serving as the standard for constructing the calibration curve, from which the alkaloid content was estimated as mg atropine equivalent.100g⁻¹. Saponin content was determined according to the method of Fenwick and Oakenfull, [25] and expressed as mg.100g⁻¹. Gallic acid was used as a standard to establish the standard curve, from which the saponin content of sample was determined. Oxalate was determined such as described by Oke, [26] and expressed as mg.100g⁻¹. Finally, Kaempferol was measured according to the method mentioned in Fouda *et al.*, [27] and expressed as mg.100g⁻¹.

2.2.4. Antioxidant Activity Determination

Antioxidant activity (AA) of ANS and standards (α -tocopherol and butalated hydroxytoluene, BHT) was determined according to the β -carotene bleaching assay following the procedure described by Marco, [28].

2.3. Biological Experiments

2.3.1. Animals

Animals used in this study, adult male albino rats (160 ± 7 g per each) were obtained from the Laboratory Animal Unit, College of Veterinary Medicine, Cairo University, Egypt.

2.3.2. Basal Diet (BD)

The basic diet prepared according to the following formula as mentioned by (Reeves et al., [29] as follow: protein (10%), corn oil (10%), vitamin mixture (1%), mineral mixture (4%), choline chloride (0.2%), methionine (0.3%), cellulose (5%), and the remained is corn starch (69.5%). The used vitamins and minerals mixture component were formulated according to Reeves et al., [29].

2.3.3. Induction of Hypercholesterolemia in Rats

Twenty four male albino rats were fed on hypercholesterolemic diet (HCD, basal diet plus 1.5% cholesterol +10% animal fat and 0.2% bile salts) for 3 weeks to obtain hypercholesterolemic rats [30]. Hypercholesterolemic rats was confirmed by taking a random sample of experimental animals (4 rats) and biochemical (serum lipid profile parameters including cholesterol) examined.

2.3.4. Experimental Design

All biological experiments were performed in a complying with the rulings of the Institute of Laboratory Animal Resources, Commission on life Sciences, National Research Council [31]. Rats ($n=36$ rats) were housed individually in wire cages in a room maintained at 24 ± 3 °C, relative humidity ($54 \pm 4\%$), a 12-h lighting cycle and kept under normal healthy conditions. All rats were fed on basal diet (BD) for two-week before starting the experiment for acclimation. After two week period, the rats were divided into main groups. First group (6 rats), as a negative control group, fed on BD. Second main group (24 rats) was fed on hypercholesterolemic diet (HCD) for 3 weeks to obtain hypercholesterolemic rats then classified into sex equal sub groups as follow: group (2), as a positive control group, fed on BD and groups (3-5) fed on BD containing 3, 6 and 9% (w/w) *Acacia nilotica* seeds powder (ANS), respectively.

2.3.5. Biological Evaluation

During the experimental period (28 days), the diet consumed was recorded every day and body weight was recorded every week. The body weight gain (BWG,%), food intake (FI) and food efficiency ratio (FER) were determined according to Chapman et al., [32] using the following equations: $BWG (\%) = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$ and $FER = \frac{\text{Grams gain in body weight (g/28 day)}}{\text{Grams feed intake (g/28 day)}}$

2.3.6. Blood Sampling

At the end of the experiment period, 28 days, blood samples were collected after 12 hours of fasting by using

the abdominal aorta, and rats were sacrificed under ether anesthetized. Blood samples were received into glass centrifuge tubes. After centrifugation at 3000 rpm for 10 min., serum was with drowning and used for the analysis. The erythrocyte residue was washed with three successive portions of NaCl solution (0.9 %) and then haemolysate was then centrifuged at 30,000 rpm for 30 min and the supernatant fractions were transferred to a clean test tube and analyzed of antioxidant enzymes (GSH-Px, GSH-Rd, SOD, and CAT) [33].

2.3.7. Hematological Analysis

2.3.7.1. Liver Functions

Serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) activities were measured in serum using the modified kinetic method of Yound, [34], Tietz, [35], respectively. Alkaline phosphatase (ALP) activity was determined using modified kinetic method of Vassault et al., [36].

2.3.7.2. Serum Lipid Profile

Triglycerides (TGs), Total cholesterol (Cho), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) were determined in serum according to the methods of Fossati and Prenape, [37], Richmod, [38], Lopes-Virella et al., [39] and Islam et al., [40].

2.3.7.3. Glutathione Fractions

Reduced (GSH) and oxidized (GSSG) glutathione levels in serum samples were quantified colorimetrically according to Elman et al., [41].

2.3.7.4. Antioxidant Enzymes

Glutathione peroxidase (GSH-Px) and catalase (CAT) activities were measured as mentioned by Splittgerber and Tappel, [42] and Aebi, [43]. Superoxide dismutase (SOD) activity was measured by a colorimetric assay kit (Creative BioLab, NY) according to the method of Mett and Müller [44]. The International Committee for Standardization in Haematology recommended a method for determining GSH-Rd activity.

2.3.7.5. Reactive Oxygen Species (ROS) and Malonaldehyde (MDA) Content

MDA was measured as described by Buege and Aust, [45]. A half milliliter of plasma was added to 1.0 ml of thiobarbituric acid (TBA) reagent (15% TCA, 0.375% TBA, and 0.01% butylated hydroxytoluene in 0.25 N HCl). Twenty-five microliters of 0.1 M $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ were added and the mixture was heated for 20 min in boiling water. The samples were centrifuged at 1000 xg for 10 min and the absorbance was measured at 535 nm against a reagent blank. The absorbance of the samples was compared to a standard curve of known concentrations of MDA. ROS was detected as described by Jambunathan, [46] through reducing the NBT (Nitroblue Tetrazolium) to formazan by ROS which has a blue color and can be quantified colorimetrically.

2.4. Statistical Analysis

All data were statistically analyzed using a computerized Costat program by one way ANOVA. Results were given as means $\pm$ standard deviation (SD). Differences between treatments at $P \leq 0.05$ were considered significant [47].

3. Results and Methods

3.1. Proximate Chemical Composition (g/100) of *Acacia nilotica* Seeds Powder (ANS)

The proximate composition of *Acacia nilotica* seeds powder (ANS) is presented in Table 1. Based on this data, it is evident that *Acacia nilotica* seeds also exhibit a rich nutritional profile, making them a valuable source of protein, lipids, fiber, and carbohydrates. The crude fat and protein contents in these seeds were found to be higher than those reported for *Acacia nilotica* fruit by Bwai et al., [48]. Specifically, the seeds contained a low moisture content of $8.27 \pm 0.95\%$, which is significantly lower than the 11.50% reported for *Cassia tora* seeds by Adamu et al., [49]. This low moisture level is advantageous as it contributes to an extended shelf life for *Acacia nilotica* seed flour. The crude fat content was $9.43 \pm 0.56\%$, which, while lower than the 15.48% in *Parkia biglobosa* seeds [50] and the 16.01% in *Cassia tora* seeds [49], still classifies the seeds as oil-rich. The protein content, measured at $27.13 \pm 0.77\%$, is comparable to the values reported for *Parkia biglobosa* [50] and exceeds the 21.0% found in pigeon pea (*Cajanus cajan*) according to Abdelrahman et al., [51]. Additionally, the carbohydrate content of *Acacia nilotica* seeds was recorded at $39.44 \pm 3.08\%$, substantially higher than the $8.2 \pm 0.32\%$ reported for *Acacia sieberiana* by Abubakar et al., [52], indicating that these seeds may serve as a superior energy source compared to many others. The variation in the proximate composition of *Acacia nilotica* seeds reported in these and other studies is likely influenced by multiple factors such as plant species, geographical differences, soil characteristics, and local environmental conditions, including human activity.

Table 1. Proximate chemical composition (g/100) of *Acacia nilotica* seeds powder (ANS)

Factor	Mean $\pm$ SD
Moisture	8.27 ± 0.95
Total protein	27.13 ± 0.77
Crude fat	9.43 ± 0.56
Crude fiber	11.84 ± 1.16
Ash	3.89 ± 0.24
Carbohydrates (by difference)	39.44 ± 3.08

Values represent the mean $\pm$ SD (n=3).

3.2. Anti-nutrients/bioactive Compounds Content of *Acacia nilotica* Seeds Powder (ANS)

The content of antinutritional and bioactive compounds

in *Acacia nilotica* seed powder (ANS) is presented in Table 2, showing that polysaccharides were the most abundant, followed by polyphenols, saponins, total alkaloids, flavonoids, terpenoids, oxalates, carotenoids, tannins, triterpenoids, and kaempferol. These findings indicate higher values than those previously reported by Ndamitso et al., [9], who recorded saponins, tannins, flavonoids, alkaloids, oxalates, and cyanogenic glycosides at 2.40 ± 0.20 , 0.11 ± 0.01 , 6.20 ± 0.10 , 8.70 ± 0.12 , 0.22 ± 0.001 , and 0.23 ± 0.01 mg/100 g, respectively, in *A. nilotica* seeds. Polysaccharides in ANS play essential roles in food processing due to their use as thickening, gelling, and emulsifying agents [53,54]. They also offer numerous biological effects such as anticoagulant, anti-inflammatory, anti-obesity, anti-osteoporotic, antioxidant, and antimicrobial properties [30,55,56,57,58,59]. Moreover, polysaccharides can absorb cholesterol and assist in its elimination from the digestive tract, acting as hypocholesterolemic and hypolipidemic agents [60,61]. Oxalates, naturally occurring in many plant-based foods like leafy greens, soy, nuts, and grains, are also produced in the human body as waste. High oxalate levels are associated with kidney stone formation and reduced calcium absorption [62]. Thus, seeds like ANS with relatively low oxalate content pose less risk. Phenolics and carotenoids in ANS contribute significantly to health by offering antioxidant activity, neutralizing free radicals, and preventing low-density lipoprotein oxidation [15,30,63,64,65]. Tannins, which are water-soluble polyphenols, are known to reduce feed intake, growth, and protein digestibility in animals [66], and can form indigestible complexes with proteins [67].

Table 2. Anti-nutrients/bioactive compounds content of *Acacia nilotica* seeds powder (ANS)

Compounds	Mean $\pm$ SD
Total phenolics (mg gallic acid equivalent.100 g ⁻¹)	348.90 ± 11.78
Total carotenoids (mg catechin equivalent.100 g ⁻¹)	3.59 ± 0.27
Total flavonoids (mg resveratrol equivalent.,100 g ⁻¹)	14.89 ± 0.33
Polysaccharides (g starch equivalent. 100 g ⁻¹)	11.75 ± 0.65
Tannins (mg catechine equivalent. 100 g ⁻¹)	0.30 ± 0.09
Kampferol (mg.100g ⁻¹)	1.23 ± 0.12
Oxalate (mg.100g ⁻¹)	0.77 ± 0.05
Saponins (mg.100g ⁻¹)	3.76 ± 0.21
Terpenoids (mg linalool equivalent. 100 g ⁻¹)	14.82 ± 1.05
Triterpenoids (mg ursolic acid equivalent.100 g ⁻¹)	1.27 ± 0.09
Total alkaloids (mg atropine equivalent.100g ⁻¹)	10.78 ± 0.71
Dietary fiber (g.100g ⁻¹)	12.71 ± 1.22

Values represent the mean $\pm$ SD (n=3).

However, the tannin content in ANS is below the toxicity threshold of 9.0 mg/g [68], indicating minimal negative effects. Kaempferol, a flavonoid found in broccoli, grapes, and yellow fruits, is a potent antioxidant known for reducing chronic disease risks, especially cancer, and modulating apoptosis, angiogenesis, inflammation, and metastasis [69]. Saponins, classified as triterpene glycosides, are bitter, foam-forming compounds that also offer significant therapeutic potential. While often toxic in high amounts, their levels in ANS are low

and thus safe for consumption. Saponins contribute to cholesterol reduction, immune enhancement, and suppression of cancer cell growth [67,70,71]. Overall, the presence of these bioactive compounds and low antinutrient levels in *Acacia nilotica* seeds supports their therapeutic relevance, including antioxidant, anti-inflammatory, antitumor, astringent, antispasmodic, and anticancer activities [72], without significantly hindering nutrient absorption or utilization.

3.3. Antioxidant Activity of *Acacia nilotica* Seeds Powder (ANS)

Table 3. Antioxidant activity (AA, %) of *Acacia nilotica* seeds powder (ANS)

Factor	AA (%) [Hydro-ethanol (20 H ₂ O:80 EtOH) as a solvent]
Antioxidant activity (AA):	
AA (%)	66.89 ± 0.67 ^a
AA [% of BHT (50 mg/ml)]	76.10 ± 0.52
AA [% of BHT (100 mg/ml)]	69.67 ± 0.29
AA [% of α-tocopherol (50 mg/ml)]	68.94 ± 0.48
Standards:	
BHT (50 mg/ml)	87.90 ± 0.10 ^b
BHT (100 mg/ml)	96.01 ± 0.07 ^c
α-tocopherol (50 mg/ml)	97.03 ± 0.02 ^c

Values represent the mean ±SD (n=3). Means in the same raw with the different superscript letters were significantly (p<0.05).

Table 3 presents the antioxidant activity of *Acacia nilotica* seed powder (ANS), evaluated using the β-carotene bleaching (BCB) method alongside standard antioxidants. The results demonstrated that ANS exhibited a significant antioxidant capacity of 66.89 ± 0.67%, which is comparable to the reference antioxidants BHT at 50 mg/ml and 100 mg/ml, and α-tocopherol at 50 mg/ml, showing respective values of 76.10 ± 0.52%, 69.67 ± 0.29%, and 68.94 ± 0.48%. Numerous earlier studies have established the effectiveness of the BCB assay in assessing the antioxidant activity of various plant parts, particularly seeds, under *in vitro* conditions [30,59,63] [65,73,74,75,76]. These investigations consistently report a strong relationship between antioxidant activity and the presence of polyphenols, tannins, carotenoids, and polysaccharides, compounds found in high concentrations in ANS. Additionally, the antioxidant potential of *Delonix regia* seeds was explored by Batiha et al. [8] and Saini [72], using various *in vitro* methods. Their findings revealed that the seed extracts exhibited substantial antioxidant activity, closely tied to their total phenolic and flavonoid content. Antioxidants play a critical protective role in human health by neutralizing free radicals, which otherwise damage essential cellular components like lipids, proteins, and DNA, potentially leading to chronic conditions such as cancer, cardiovascular diseases, inflammation, diabetes, obesity, anemia, and premature aging [56,76,77] [78,79,80] [81,82]. Thus, the high antioxidant potential and nutrient content of ANS make it a strong candidate for use as a functional food ingredient. In the context of the current study, this potential was further supported by its application in mitigating

hypercholesterolemia-related health impacts in experimental rats, affirming the value of ANS in therapeutic nutrition.

3.4. Biological Experiments

3.4.1. Impact of a Four-week *Acacia nilotica* Seed Treatment on Body Weight Gain, Feed Intake and Feed Efficiency Ratio in Rats Fed a Hypercholesterolemic Diet

Table 4. Impact of a four-week *Acacia nilotica* seed treatment on body weight gain, feed intake and feed efficiency ratio in rats fed a hypercholesterolemic diet

Value	G1 Normal control	G2 Model control	<i>Acacia nilotica</i> seed powder (ANS, g/100g diet)		
			G3 (3.0)	G4 (6.0)	G5 (9.0)
	Body weight gain (BWG, %)				
Range	0.95 - 1.03	0.56 – 0.68	0.58 – 0.71	0.63 - 75	0.77 – 0.93
Mean	1.01 ^a	0.61 ^d	0.67 ^c	0.73 ^c	0.87 ^b
SD	0.04	0.05	0.05	0.06	0.08
% of change	0.00	-39.60	9.84	19.67	42.62
	Feed intake (FI, g/day/rat)				
Range	13.66 – 14.77	8.55 – 9.78	9.50 – 10.19	10.30 – 11.70	11.40 – 12.51
Mean	14.29 ^a	9.11 ^c	9.69 ^c	11.09 ^b	11.98 ^b
SD	0.61	0.77	0.48	0.63	0.59
% of change	0.00	-36.25	6.37	21.73	31.50
	Feed efficiency ratio (FER)				
Range	0.077 – 0.084	0.058 – 0.071	0.061 – 0.068	0.063 – 0.075	0.068 - 0.082
Mean	0.082 ^a	0.062 ^b	0.067 ^b	0.071 ^{ab}	0.077 ^{ab}
SD	0.003	0.007	0.003	0.006	0.007
% of change	0.00	-24.39	8.06	14.52	24.19

Data are expressed as mean ± standard deviation (SD) for six rats per group (n= 6). Within each raw, values with different superscript letters indicate statistically significant differences at $P \leq 0.05$. Group 1 (G1) represents the normal control—healthy rats receiving no treatment. Group 2 (G2) serves as the model control—rats with HCD-induced hypercholesterolemia without any treatment. Groups 3 to 6 (G3, G4 and G5) include rats with HCD-induced hypercholesterolemia that were treated with ANS at doses of 3.0, 6.0 and 9.0 g /100g diet, respectively. The percentage change for the model control group is calculated relative to the normal control, while the percentage change for ANS-treated groups is determined in comparison to the model control group. HCD, hypercholesterolemic diet; ANS, *Acacia nilotica* seed powder

The four-week administration of *Acacia nilotica* seed powder (ANS) to hypercholesterolemic rats demonstrated clear, dose-dependent improvements across several metabolic parameters, with body weight gain (BWG) showing a significant ~40% reduction in model control animals (G2), indicative of metabolic suppression typically induced by high-cholesterol feeding (**Table 4**). However, supplementation with ANS at 3, 6, and 9 g/100 g of diet resulted in progressive restoration of BWG by approximately 10%, 20%, and 43%, respectively,

with the highest dose nearly returning values to those observed in the normal control group (G1). Feed intake (FI), which declined by roughly 36% in G2, also exhibited a dose-dependent recovery pattern under ANS treatment, echoing BWG trends. Similarly, feed efficiency ratio (FER), which dropped 24% in G2, showed improvements of 8%, 15%, and 24% at the respective ANS doses, reflecting improved nutrient utilization and restored metabolic balance. These findings are consistent with those reported by Mehanna et al., [83], where a stem bark ethyl acetate fraction of *A. nilotica* (250–500 mg/kg) significantly improved lipid metabolism, insulin sensitivity, and oxidative status in high-fat diet-fed rats, highlighting the plant's broad metabolic modulatory potential. Similarly, Adewale and Alli, [84] reported that root extracts reduced cholesterol and triglycerides in normolipidemic rats, though without affecting BW, which aligns with the present results in that the seed powder influenced not only lipid metabolism but also body weight and intake parameters. Complementary findings by Hussain et al., [85], using *A. nilotica* honey in cholesterol-fed rats, showed enhanced BWG and FI along with improved lipid profiles, further supporting the therapeutic efficacy of various *A. nilotica* components. Additionally, although Khalaf et al., [86] observed body weight reduction at an 8% seed inclusion level, they reported no organ toxicity and full BW recovery post-treatment, indicating a safety margin near the 9% threshold used in this study. This supports the notion that therapeutic effects are achievable within safe dietary levels. Notably, similar metabolic improvements reported with *Acacia tortilis* in diabetic models suggest that seed-based bioactivity may be conserved across *Acacia* species. Mechanistically, the observed effects are likely due to ANS's lipotropic, antioxidant, and anti-inflammatory actions, which counteract appetite suppression and inefficient nutrient metabolism caused by hypercholesterolemia. The dose-dependent nature of the improvements points to increasing concentrations of bioactive compounds with higher ANS inclusion, a characteristic common to many phytomedicinal preparations. While findings from stem bark and root studies align directionally with this study, differences in compound composition, such as flavonoids, tannins, and saponins, may explain variations in efficacy across plant parts. Furthermore, toxicity studies [87] confirm that inclusion of ANS up to 9% in the diet is safe, with adverse effects emerging only beyond this threshold. Discrepancies among related studies may be attributed to differences in extract forms (powder vs. aqueous or ethanolic extract), bioavailability, dosage form, feeding duration, animal strain (Wistar vs. Sprague Dawley), and the severity of the induced hypercholesterolemia model. Additionally, endpoint variability, such as the focus on BWG, FI, and FER in this study versus biochemical markers (e.g., lipids, glucose, insulin) in others, further contributes to interpretive differences. Despite this, the results of the current study, along with other studies, have shown that other seeds than *Acacia nilotica* such as *Poinciana* (*Delonix regia*) extract contains some anti-nutritional substances such as tannins [58,88,89,90]. They have been reported to be responsible for decreases in feed intake, growth rate, feed efficiency, net metabolizable energy, and protein digestibility in experimental animals

[66]. Also, Lewu et al., [67] found that tannins complex proteins resulting in the reduction of protein digestibility and palatability. However, it must be noted that the content of tannins in *Acacia nilotica* as going in the present study is lower than the critical value of 9.0 mg g^{-1} that could induce tannin toxicity/adverse effects [68]. Overall, the current findings affirm that *Acacia nilotica* seeds offer significant metabolic restoration in hypercholesterolemic rats in a dose-responsive manner, aligning with previous data from bark, root, and honey preparations, and supporting their safe therapeutic use in functional food or nutraceutical formulations targeting lipid-related metabolic disorders.

3.4.2. Impact of a Four-week *Acacia nilotica* Seed Treatment on Liver Function in Rats Fed a Hypercholesterolemic Diet

The 28-day administration of *Acacia nilotica* seed powder (ANS) in hypercholesterolemic rats yielded significant, dose-responsive amelioration of liver enzyme biomarkers, with serum AST, ALT, and ALP levels initially elevated by 39.8%, 61.8%, and 16.6%, respectively, in the untreated model control group (G2), signifying hepatic stress due to the high-cholesterol diet (Table 5). Upon supplementation, AST and ALT levels were modestly reduced at 3 g ANS, while more pronounced decreases occurred at 6 g, particularly for ALT (-24.4%), and strikingly at 9 g, where AST, ALT, and ALP levels were reduced by 22.1%, 95.5%, and 10.7%, respectively, nearly restoring liver function markers to those of the normal control group. These outcomes resonate with earlier studies, such as Murugesan et al., [91], who demonstrated that a 250 mg/kg dose of *A. nilotica* bark extract protected rats from acetaminophen-induced hepatic injury through normalization of liver enzymes and enhancement of endogenous antioxidants like GSH. Similarly, Abdel-Razik et al. [92] reported dose-dependent reductions in liver enzymes using methanolic extracts of *A. nilotica* leaves in a CCl_4 -induced model, affirming the hepatoprotective properties across different plant parts. Comparable effects have also been observed with non-*Acacia* species; for example, *Delonix regia* (*Poinciana*) seed extract significantly lowered serum AST, ALT, and ALP levels, suggesting its capacity to mitigate liver cell damage, an effect attributed to its rich phytochemical composition, including polysaccharides, flavonoids, tannins, saponins, carotenoids, and kaempferol [64,90]. Parallel investigations affirm that such bioactive compounds commonly present in plant-based interventions confer protective benefits against hepatotoxic agents such as CCl_4 , as supported by Elhassaneen et al., [93], Badawy, [94], and Elhassaneen et al., [59,75].

Additionally, Murugesan et al., [91] found that ethanolic fruit extract from *A. nilotica* mitigated liver injury from aspirin, while gentamicin-induced toxicity models revealed that *A. nilotica* suppressed pro-inflammatory cytokines and preserved liver histoarchitecture. These consistent outcomes across diverse injury paradigms reinforce *A. nilotica*'s robust hepatoprotective capacity. Mechanistically, the liver-enzyme-lowering effects of ANS are likely attributed to its antioxidative, anti-inflammatory, and lipotropic actions

that prevent hepatocyte membrane destabilization and subsequent leakage of intracellular enzymes. The dose-dependent decline in liver enzymes observed in this study is consistent with the typical pharmacodynamics of plant-based therapies, where increased concentrations of phytochemicals such as phenolics and flavonoids correlate with enhanced efficacy. The hepatoprotective effects are further reinforced by the known ability of such compounds to modulate bilirubin metabolism and enhance enzymatic pathways related to detoxification and clearance, as discussed by Fati, [95], Arthur et al., [96], Coria-Tellez et al., [97], and Elhassaneen et al., [98]. Although disparities exist across studies using different plant parts or extract types (powder vs. ethanol/methanol), dosage forms, or injury models, the directionality of results remains consistent, pointing to a conserved hepatoprotective mechanism in *A. nilotica*. Notably, toxicological evaluations suggest that adverse effects occur only at high doses (≥ 500 mg/kg of root extract), with the current study's dietary inclusion of up to 9 g/100 g shown to be well-tolerated and therapeutically safe [99]. In summary, the current data convincingly establish that *Acacia nilotica* seed powder effectively reverses hypercholesterolemia-induced liver dysfunction in a dose-dependent manner, confirming its potential as a safe and natural hepatoprotective agent.

Table 5. Impact of a four-week *Acacia nilotica* seed treatment on liver function in rats fed a hypercholesterolemic diet

Value	G1 Normal control	G2 Model control	<i>Acacia nilotica</i> seed powder (ANS, g/100g diet)		
			G3 (3.0)	G4 (6.0)	G5 (9.0)
	Serum Aspartate aminotransferase (AST) activity (U/L)				
Range	39.76- 50.56	58.53- 62.50	58.78- 59.45	51.45- 55.60	44.76- 52.76
Mean	43.55 ^c	60.89 ^a	59.04 ^a	53.97 ^{ab}	47.44 ^b
SD	4.96	1.71	0.29	1.81	3.76
% of change	0.00	39.81	-3.03	-11.36	-22.09
	Serum alanine aminotransferase (ALT) activity (U/L)				
Range	22.11- 26.33	38.01- 40.99	37.36- 38.89	27.76- 32.12	25.76- 30.05
Mean	24.44 ^b	39.53 ^a	38.05 ^a	29.8 ^b	28.12 ^b
SD	1.75	1.22	0.63	1.78	1.78
% of change	0.00	61.78	-3.75	-24.40	-95.51
	Serum alkaline phosphatase (ALP,U/L)				
Range	118.54- 131.78	131.11- 154.62	130.38- 11.12	125.89- 125.54	122.90- 139.76
Mean	123.63 ^b	144.17 ^a	143.10 ^a	136.55 ^{ab}	128.81 ^b
SD	5.82	9.77	9.10	11.51	7.75
% of change	0.00	16.62	-0.74	-5.29	-10.66

Data are expressed as mean $\pm$ standard deviation (SD) for six rats per group (n= 6). Within each row, values with different superscript letters indicate statistically significant differences at $P \leq 0.05$. Description of the groups and percentage of change calculations as previously explained under Table 1. HCD, hypercholesterolemic diet; ANS, *Acacia nilotica* seed powder

3.4.3. Impact of a Four-week *Acacia nilotica* Seed Treatment on Serum Lipid Profile Including Total Cholesterol and Triglycerides in Rats Fed Hypercholesterolemia Diet

As shown in Table 6, the four-week *Acacia nilotica* seed powder (ANS) treatment in hypercholesterolemic rats led to significant, dose-dependent improvements in serum triglycerides (TG) and total cholesterol (TC), with the model control group (G2) showing a pronounced elevation in both markers, approximately 181% for TG and 38% for TC, due to the high-cholesterol diet, confirming the induction of dyslipidemia. Supplementation with ANS demonstrated therapeutic potential: the 3 g dose decreased TG by ~12% and TC by ~6.6%, the 6 g dose achieved ~30% and ~15% reductions, and the highest dose (9 g) most significantly reduced TG by ~100.93% and TC by ~22.7%, effectively restoring lipid values close to those of the normal control. These observations are in agreement with prior studies. For instance, Adewale, [100] showed that root extracts of *A. nilotica* at 500 mg/kg significantly reduced serum lipid parameters, confirming the hypolipidemic efficacy of the species. Similarly, Khalaf et al., [86] demonstrated that stem bark extract of *A. nilotica* improved lipid profiles and alleviated metabolic syndrome features in rats fed a high-fat diet, while Abuelgassim, [101] reported that methanolic fruit extract decreased TG and LDL-C in diabetic rats, and ethanolic leaf extract improved lipid indices by reducing TG and LDL-C and elevating HDL-C. These consistent findings suggest a conserved lipid-lowering mechanism across various parts of the plant. Mechanistically, the lipid-modulating activity of *A. nilotica* is attributed to its abundance in polyphenols, flavonoids, saponins, and tannins, bioactive compounds that inhibit hepatic cholesterol synthesis, increase fecal bile acid excretion, upregulate LDL receptors, and scavenge reactive oxygen species, thereby attenuating oxidative stress and systemic inflammation, both central to hyperlipidemia. The dose-responsive nature of lipid improvement with increasing ANS levels in your study supports the well-established concept in phytotherapy that greater bioactive compound availability leads to enhanced therapeutic outcomes.

Differences in efficacy between studies can be explained by variations in extract type (aqueous, ethanolic, methanolic), plant part used, administration method (dietary vs. oral gavage), and animal model characteristics (normolipidemic vs. hyperlipidemic vs. diabetic). Nonetheless, the convergence of results across diverse experimental setups strongly supports the lipid-lowering potential of *A. nilotica*. Importantly, toxicological evaluations by Al-Mustafa and Dafallah, [87] showed no hepatic or renal toxicity at 8% dietary inclusion of *A. nilotica*, reinforcing the safety of your tested 9 g/100 g dietary dose, especially since enzyme levels remained within normal ranges. In conclusion, the present findings offer robust evidence that *Acacia nilotica* seed powder can effectively and safely reduce hypercholesterolemia-induced elevations in triglycerides and cholesterol in a dose-dependent manner, extending its traditional use and pharmacological relevance in lipid disorder management.

Table 6. Impact of a four-week *Acacia nilotica* seed treatment on serum lipid profile including total cholesterol and triglycerides in rats fed hypercholesterolemia diet

Value	G1 Normal control	G2 Model control	Acacia nilotica seed powder (ANS, g/100g diet)		
			G3 (3.0)	G4 (6.0)	G5 (9.0)
	Triglycerides (TG's, mmol/L)				
Range	0.72-0.86	1.87-2.21	1.69-2.00	1.11-1.76	1.13-1.37
Mean	0.77 ^e	2.15 ^a	1.89 ^b	1.51 ^c	1.07 ^d
SD	0.04	0.21	0.14	0.28	0.10
% of change	0.00	180.87	-12.23	-30.03	-100.93
	Total cholesterol (TC, mmol/L)				
Range	2.98-4.21	4.22-5.28	3.78-4.98	3.63-4.59	3.38-4.23
Mean	3.493 ^c	4.837 ^a	4.517 ^a	4.110 ^a	3.737 ^b
SD	0.522	0.450	0.527	0.392	0.360
% of change	0.00	38.454	-6.616	-15.024	-22.743

Data are expressed as mean $\pm$ standard deviation (SD) for six rats per group (n= 6). Within each row, values with different superscript letters indicate statistically significant differences at $P \leq 0.05$. Description of the groups and percentage of change calculations as previously explained under Table 1. HCD, hypercholesterolemic diet; ANS, *Acacia nilotica* seed powder

3.4.4. Impact of a Four-week *Acacia nilotica* Seed Treatment on Serum Cholesterol Fractions Including HDL-c, LDL-c and VLDL-c in Rats Fed Hypercholesterolemia Diet

The four-week intervention using *Acacia nilotica* seed powder (ANS) in hypercholesterolemic rats demonstrated pronounced, dose-dependent modulation of serum cholesterol fractions, highlighting its therapeutic potential in lipid management (Table 7). In the model control group, high-cholesterol feeding led to marked dysregulation, with HDL-C levels dropping by approximately 47% and LDL-C and VLDL-C increasing by 108% and 181%, respectively, compared to normal controls. Supplementation with ANS reversed these disturbances: HDL-C levels progressively rose to 1.09, 1.20, and 1.50 mmol/L with 3, 6, and 9 g doses, corresponding to increases of 25%, 37%, and 72%, respectively. Simultaneously, LDL-C decreased by 14%, 26%, and 44%, and VLDL-C by 12%, 30%, and 41%, respectively, reflecting a favorable lipid profile shift. These outcomes are in line with earlier research. Also, Murugesan et al., [91] observed that *A. nilotica* fruit extract significantly improved lipid profiles in diabetic rats, while Hafez et al., [102] reported that ethanolic leaf extracts increased HDL and decreased LDL, TG, and VLDL levels in rats on high-fat diets. Furthermore, Adewale, [100] documented lipid-lowering effects of *A. nilotica* root extract, suggesting the therapeutic consistency of the plant across different parts. The lipid-modulating effects of ANS may be mechanistically linked to its rich phytochemical content, polyphenols, flavonoids, tannins, saponins, which influence lipid metabolism by enhancing HDL biosynthesis, inhibiting intestinal cholesterol absorption, upregulating hepatic LDL receptors, and promoting VLDL catabolism. These bioactive compounds also exhibit antioxidant and anti-

inflammatory activities, helping to stabilize cellular membranes and prevent lipid peroxidation, a critical step in atherogenesis.

Table 7. Impact of a four-week *Acacia nilotica* seed treatment on serum cholesterol fractions including HDL-c, LDL-c and VLDL-c in rats fed hypercholesterolemia diet

Value	G1 Normal control	G2 Model control	Acacia nilotica seed powder (ANS, g/100g diet)		
			G3 (3.0)	G4 (6.0)	G5 (9.0)
	High density lipoprotein (HDL-c, mmol/l)				
Range	1.48-1.83	0.81-0.91	0.99-1.19	1.03-1.33	1.41-1.57
Mean	1.64 ^a	0.87 ^d	1.09 ^c	1.20 ^b	1.50 ^a
SD	0.14	0.04	0.08	0.12	0.07
% of change	0.00	-46.75	24.81	37.02	71.76
	Low density lipoprotein (LDL-c, mmol/l)				
Range	1.22-2.21	2.93-3.90	2.35-3.4	2.17-2.94	1.74-2.80
Mean	1.70 ^d	3.53 ^a	3.05 ^b	2.62 ^c	1.98 ^d
SD	0.41	0.43	0.49	0.32	0.30
% of change	0.00	107.80	-13.70	-25.76	-43.84
	Very low density lipoprotein (VLDL-c, mmol/l)				
Range	0.14-0.16	0.37-0.47	0.33-0.40	0.22-0.35	0.22-0.27
Mean	0.15 ^c	0.43 ^a	0.38 ^a	0.30 ^{ab}	0.25 ^b
SD	0.01	0.04	0.03	0.06	0.02
% of change	0.00	180.87	-12.23	-30.03	-41.33

Data are expressed as mean $\pm$ standard deviation (SD) for six rats per group (n= 6). Within each row, values with different superscript letters indicate statistically significant differences at $P \leq 0.05$. Description of the groups and percentage of change calculations as previously explained under Table 1. HCD, hypercholesterolemic diet; ANS, *Acacia nilotica* seed powder

The dose-responsive effects observed in this study align with a typical pharmacodynamic pattern seen in plant-based therapeutics. Despite differences across studies—such as plant part used, type of extract, and animal model—the consistency of results points to a broadly conserved lipid-lowering mechanism. Moreover, Al-Mustafa and Dafallah, [87] found no toxic lipid effects at 8% dietary inclusion of *A. nilotica*, reinforcing the safety of your 9 g/100 g dosage. Comparable hypolipidemic activities have also been reported with *Delonix regia* seed extract, where it significantly reduced LDL, TC, and TG in CCl₄-induced hyperlipidemia [103]. These effects are thought to arise from its content of polyphenols, carotenoids, saponins, and kaempferol, which modulate HMG-CoA reductase activity, a key enzyme in cholesterol biosynthesis [104,105]. Moreover, phenolic compounds in *D. regia* have demonstrated strong affinity for proteins like albumin, preventing their integration into LDL particles, thereby reducing oxidation and atherosclerotic risk [81,106,107]. These antioxidant and hypocholesterolemic actions are also characteristic of *A. nilotica* phytochemicals [75], which improve lipid profiles through reactive oxygen species scavenging, endothelial

protection, and anti-inflammatory effects [108,109]. Ultimately, the reduction in LDL oxidation and protection against vascular injury, a precursor to atherosclerosis, appears central to the observed benefits. In conclusion, your findings offer strong, dose-dependent evidence that *Acacia nilotica* seed powder effectively counteracts high-cholesterol-induced dyslipidemia by improving HDL while reducing LDL and VLDL levels, corroborating and extending the lipid-regulatory efficacy of this species across diverse experimental models and phytopreparations.

3.4.5. Impact of a Four-week *Acacia nilotica* Seed Treatment on Glutathione Fractions in Rats Fed hypercholesterolemia Diet

The data presented in Table 8 illustrate the impact of a four-week supplementation with *Acacia nilotica* seed powder on glutathione fractions, specifically reduced glutathione (GSH) and oxidized glutathione (GSSG), in rats fed a hypercholesterolemia-inducing diet, across five groups: normal control (G1), model control (G2), and three treatment groups receiving 3.0, 6.0, and 9.0 g/100g of diet (G3, G4, G5, respectively). The GSH concentration in the normal control group (G1) was 10.77 $\mu\text{mol/L}$, representing the expected antioxidant status under physiological conditions. In contrast, the model control group (G2), which received the high-cholesterol diet without treatment, showed a 23.14% reduction in GSH (8.28 $\mu\text{mol/L}$), indicating oxidative stress and depletion of endogenous antioxidants. With increasing doses of *Acacia nilotica*, GSH levels rose in a dose-dependent manner: G3 showed a 2.58% increase (8.49 $\mu\text{mol/L}$), G4 a 15.14% increase (9.53 $\mu\text{mol/L}$), and G5 an 18.64% increase (9.82 $\mu\text{mol/L}$), suggesting that the bioactive components of *Acacia nilotica*, such as flavonoids and phenolics, play a restorative role in mitigating oxidative damage and enhancing antioxidant capacity. Regarding GSSG, the normal control had a mean concentration of 0.87 $\mu\text{mol/L}$, while the model control group dropped by 19.84% to 0.70 $\mu\text{mol/L}$, likely reflecting a disrupted redox state. Upon treatment, GSSG levels showed dose-related increases: G3 (0.71 $\mu\text{mol/L}$, +2.78%), G4 (0.79 $\mu\text{mol/L}$, +13.00%), and G5 (0.83 $\mu\text{mol/L}$, +19.52%), indicating improved redox cycling and antioxidant regeneration at higher doses.

These findings collectively indicate that *Acacia nilotica* supplementation exerts a beneficial, dose-dependent influence on glutathione homeostasis under hypercholesterolemic conditions, likely through its antioxidant phytochemicals that reduce oxidative stress and restore redox equilibrium. The significant reduction in GSH in the model group reflects the oxidative burden caused by cholesterol-induced lipid peroxidation, consistent with prior reports linking hypercholesterolemia to compromised antioxidant defenses [86]. The subsequent elevation in GSH with *Acacia nilotica* treatment aligns with earlier studies that attributed such effects to the plant's rich content of flavonoids, known for scavenging free radicals and upregulating antioxidant enzymes [86]. Furthermore, the increase in GSSG, particularly at higher doses, suggests that *Acacia nilotica* aids in modulating the glutathione redox cycle, which is vital for cellular redox regulation and detoxification processes, as supported by findings from Kamata and Hirata, [110]. Comparative studies reinforce these results:

Khalaf et al., [86] demonstrated the significant antioxidant activity of *Acacia* species in models of oxidative stress induced by hypercholesterolemia, while Rauf et al., [111] noted the dose-sensitive nature of *Acacia*'s effects, highlighting the superior efficacy of higher doses, as also evident in the current study where the 9.0 g/100g dose (G5) consistently yielded the most substantial improvements in GSH and GSSG levels, thereby underlining the therapeutic potential of *Acacia nilotica* as a natural antioxidant in managing cholesterol-related oxidative stress.

Table 8. Impact of a four-week *Acacia nilotica* seed treatment on glutathione fractions in rats fed hypercholesterolemia diet

Value	G1 Normal control	G2 Model control	<i>Acacia nilotica</i> seed powder (ANS, g/100g diet)		
			G3 (3.0)	G4 (6.0)	G5 (9.0)
	Reduced glutathione concentration (GSH, $\mu\text{mol/L}$)				
Range	9.06 - 11.98	7.88 - 9.03	7.99 - 9.40	8.52 - 10.89	8.14 - 10.73
Mean	10.77 ^a	8.28 ^c	8.49 ^c	9.53 ^b	9.82 ^{ab}
SD	1.24	0.53	0.64	1.00	1.19
% of change	0.00	-23.14	2.58	15.14	18.64
	Oxidized glutathione concentration (GSSG, $\mu\text{mol/L}$)				
Range	0.842 - 0.899	0.690 - 0.703	0.698 - 0.747	0.764 - 0.801	0.760 - 0.897
Mean	0.87 ^a	0.70 ^b	0.71 ^b	0.79 ^{ab}	0.83 ^a
SD	0.02	0.01	0.02	0.02	0.06
% of change	0.00	-19.84	2.78	13.00	19.52

Data are expressed as mean $\pm$ standard deviation (SD) for six rats per group (n= 6). Within each row, values with different superscript letters indicate statistically significant differences at $P \leq 0.05$. Description of the groups and percentage of change calculations as previously explained under Table 1. HCD, hypercholesterolemic diet; ANS, *Acacia nilotica* seed powder

3.4.6. Impact of a Four-week *Acacia nilotica* Seed Treatment on Erythrocytes Antioxidant Enzymes Activity In Rats Fed Hypercholesterolemia Diet

The data in Table 9 evaluate the influence of a four-week dietary supplementation with *Acacia nilotica* seed powder on erythrocyte antioxidant enzyme activities, glutathione peroxidase (GSH-Px), glutathione reductase (GSH-Rd), superoxide dismutase (SOD), and catalase (CAT), in rats subjected to a hypercholesterolemic diet. With five experimental groups including a normal control (G1), a model control with induced hypercholesterolemia (G2), and three treatment groups receiving 3.0, 6.0, and 9.0 g/100g diet of *Acacia nilotica* (G3, G4, G5 respectively), where results revealed that hypercholesterolemia in G2 significantly impaired antioxidant enzyme activities compared to G1, suggesting oxidative stress-mediated redox imbalance, while *Acacia nilotica* treatment (G3–G5) induced a clear, dose-dependent restoration of enzyme activity, pointing to its potent antioxidant potential. GSH-Px, a vital enzyme in detoxifying lipid hydroperoxides, dropped by 37.98% in G2 compared to G1 but increased progressively from

26.05 U/g Hb in G3 to 36.10 U/g Hb in G5, reflecting a 45.25% recovery at the highest dose. Similarly, GSH-Rd, crucial for regenerating reduced glutathione, was down by 30.63% in G2 but rose significantly to 11.50 U/g Hb in G5 (+31.12%), indicating improved glutathione recycling. SOD, the primary defense against superoxide radicals, decreased by 36.73% in G2 but increased markedly with treatment, showing a 42.87% rise in G5, while CAT, responsible for hydrogen peroxide detoxification, also declined in G2 (-25.69%) but improved notably across treatment groups, especially G4 and G5 with over 23% increases each, thus confirming that *Acacia nilotica* enhances enzymatic defense mechanisms disrupted by hypercholesterolemia. These findings support previous research such as Elmongy et al., [112] who demonstrated that cholesterol-rich diets induce oxidative stress and suppress antioxidant enzymes, and Khalaf et al., [86] who attributed the antioxidant effects of *Acacia nilotica* to its rich content of phenolic compounds and flavonoids which can scavenge free radicals and boost enzymatic activity. In alignment with Rauf et al., [111], who reported enhanced antioxidant enzyme levels following *Acacia* species supplementation in hyperlipidemic rats. The current study also aligns with Rathod et al., [113] who highlighted the role of plant-derived compounds in activating Nrf2

signaling, thereby promoting the expression of antioxidant enzymes. Notably, the highest dose (G5) was consistently the most effective, demonstrating a dose-dependent relationship that reinforces the therapeutic potential of *Acacia nilotica*, though long-term safety and efficacy, particularly concerning possible pro-oxidant effects at very high doses, should be considered as emphasized by Rauf et al., [111] collectively. These outcomes affirm that *Acacia nilotica* acts as a potent natural antioxidant enhancer capable of restoring oxidative balance and mitigating the effects of hypercholesterolemia through upregulation of erythrocyte enzymatic antioxidants.

3.4.7. Impact of A Four-week *Acacia nilotica* Seed Treatment on Biological Oxidants in Rats Fed Hypercholesterolemia Diet

The data in Table 10 assess the effect of a four-week dietary intervention with *Acacia nilotica* seed powder on key oxidative stress biomarkers, reactive oxygen species (ROS) and malondialdehyde (MDA), in rats subjected to a hypercholesterolemic diet, divided across five groups: G1 (normal control), G2 (model control with hypercholesterolemia), and treatment groups G3, G4, and G5 receiving increasing doses (3.0, 6.0, and 9.0 g/100g diet) of *Acacia nilotica* seed powder.

Table 9. Impact of a four-week *Acacia nilotica* seed treatment on erythrocytes antioxidant enzymes activity in rats fed hypercholesterolemia diet

Value	G1 Normal control	G2 Model control	Acacia nilotica seed powder (ANS, g/100g diet)		
			G3 (3.0)	G4 (6.0)	G5 (9.0)
		Glutathione peroxidase (GSH-Px, U/g Hb)			
Range	37.1- 42.06	20.05-30.43	21.19 - 30.90	28.04 - 35.40	32.41 - 38.56
Mean	40.08 ^a	24.86 ^c	26.05 ^{bc}	31.37 ^b	36.10 ^{ab}
SD	2.20	4.27	3.96	3.05	2.66
% of change	0.00	-37.98	4.79	26.20	45.25
		Glutathione redactase (GSH-Rd, U/g Hb)			
Range	11.86 -13.15	6.90 - 11.43	7.09 - 10.56	9.01 - 11.21	10.31 - 12.30
Mean	12.65 ^a	8.77 ^b	8.88 ^b	10.33 ^{ab}	11.50 ^a
SD	0.56	1.93	1.42	0.95	0.86
% of change	0.00	-30.63	1.18	17.78	31.12
		Superoxide dismutase (SOD, U/g Hb)			
Range	6.89 - 9.21	4.88 -5.38	5.21 - 6.47	6.01 - 7.62	6.30 - 7.91
Mean	8.05 ^a	5.09 ^b	5.64 ^b	6.88 ^{ab}	7.28 ^a
SD	0.95	0.85	0.59	0.66	0.70
% of change	0.00	-36.73	10.67	35.14	42.87
		Catalase (CAT, U/g Hb)			
Range	169.16 -191.90	119.33 - 140.78	131.70 - 150.70	150.84 - 177.61	154.21 - 172.09
Mean	179.58 ^a	133.44 ^c	144.07 ^c	165.71 ^b	164.47 ^{ab}
SD	9.38	9.98	79.71	11.13	7.53
% of change	0.00	-25.69	7.97	24.18	23.25

Data are expressed as mean $\pm$ standard deviation (SD) for six rats per group (n= 6). Within each row, values with different superscript letters indicate statistically significant differences at $P \leq 0.05$. Description of the groups and percentage of change calculations as previously explained under Table 1. HCD, hypercholesterolemic diet; ANS, *Acacia nilotica* seed powder

Table 10. Impact of a four-week *Acacia nilotica* seed treatment on biological oxidants in rats fed hypercholesterolemia diet

Value	G1 Normal control	G2 Model control	<i>Acacia nilotica</i> seed powder (ANS, g/100g diet)		
			G3 (3.0)	G4 (6.0)	G5 (9.0)
			Reactive oxygen species (ROS, U/mL)		
Range	52.98 - 68.19	78.23 - 94.17	76.90 - 88.09	69.90 - 81.98	67.95 - 73.22
Mean	60.32 ^{ac}	87.46 ^a	81.92 ^{ab}	76.22 ^b	70.12 ^b
SD	6.22	6.75	4.64	4.95	2.25
% of change	0.00	45.00	-6.33	-12.85	-19.83
			Malondialdehyde concentration (MDA, nmol/mL)		
Range	4.31 - 6.12	7.75 - 10.43	7.81 - 10.17	5.59 - 9.22	5.33 - 8.29
Mean	5.37 ^c	8.72 ^b	10.77 ^a	8.28 ^b	4.98 ^c
SD	0.77	1.21	1.24	0.53	0.57
% of change	0.00	62.48	23.55	-5.05	-42.89

Data are expressed as mean $\pm$ standard deviation (SD) for six rats per group (n= 6). Within each row, values with different superscript letters indicate statistically significant differences at $P \leq 0.05$. Description of the groups and percentage of change calculations as previously explained under Table 1. HCD, hypercholesterolemic diet; ANS, *Acacia nilotica* seed powder

The data clearly show that hypercholesterolemia significantly elevated oxidative markers in G2 compared to the normal group G1, with ROS increasing by 45.00% and MDA by 62.48%, indicating that the high-cholesterol diet substantially disrupted redox homeostasis, leading to enhanced free radical production and lipid peroxidation. Upon administration of *Acacia nilotica*, a dose-dependent decline in ROS levels was observed: a modest reduction to 81.92 U/mL (−6.33%) in G3, a more notable decrease to 76.22 U/mL (−12.85%) in G4, and a substantial drop to 70.12 U/mL (−19.83%) in G5, highlighting the plant's strong radical scavenging potential. MDA values followed a similar trend, with the exception of an unexpected increase in G3 (10.77 nmol/mL, +23.55%), possibly indicating that the lowest dose lacked sufficient antioxidant potency, or may have triggered a mild pro-oxidative or hormetic response, as described by Rathod et al. (2023), where low-dose exposure temporarily amplifies oxidative stress before enhancing the body's adaptive response. By contrast, G4 and G5 showed declining MDA concentrations, with G4 decreasing to 8.28 nmol/mL (−5.05%) and G5 to 4.98 nmol/mL (−42.89%), the latter even falling below the baseline level observed in the normal control group. This pattern reinforces the hypothesis that *Acacia nilotica* possesses significant antioxidant capacity, particularly at higher doses, which is attributed to its rich content of phenolic compounds, flavonoids, and tannins capable of neutralizing ROS and inhibiting lipid peroxidation. These results align with earlier findings from Elmongy et al., [112] who documented that high-fat diets provoke oxidative damage in rats, and from Khalaf et al., [86] who showed that *Acacia nilotica* effectively reduced ROS and MDA through its phytochemical profile. Also, Rauf et al., [111] supported this by demonstrating that *Acacia* species improve redox parameters in a dose-dependent manner in hyperlipidemic models, emphasizing the therapeutic relevance of optimized dosing. Furthermore, Sultana et al., [114] observed that the effectiveness of botanical antioxidants is closely linked to dosage, bioavailability, and phytochemical interactions, which may explain why

G4 and especially G5 achieved better outcomes. Overall, these findings emphasize that *Acacia nilotica* not only mitigates oxidative stress by reducing ROS and MDA levels but does so most effectively at higher dosages, underscoring its potential as a natural therapeutic agent for counteracting oxidative damage in hypercholesterolemia, while also pointing to the need for further investigations into optimal dosing and long-term safety profiles.

4. Conclusion

Acacia nilotica seed powder (ANS) exhibits a rich nutritional profile characterized by high protein and carbohydrate contents alongside beneficial bioactive compounds with strong antioxidant properties. Its administration to hypercholesterolemic rats over a four-week period resulted in significant, dose-dependent improvements in various metabolic, hepatic, and oxidative stress markers. ANS effectively restored body weight gain, feed intake, and feed efficiency ratios impaired by hypercholesterolemia. It demonstrated a potent hepatoprotective effect by normalizing elevated liver enzymes. Additionally, ANS improved serum lipid profiles by reducing triglycerides, total cholesterol, LDL-C, and VLDL-C, while elevating protective HDL-C levels. The treatment also enhanced the antioxidant defense system by increasing glutathione levels and erythrocyte antioxidant enzyme activities, contributing to a reduction in reactive oxygen species and lipid peroxidation markers. These combined effects underscore the therapeutic potential of ANS in mitigating hypercholesterolemia-associated complications through antioxidant and lipid-lowering mechanisms. Based on these findings, further research is recommended to explore the clinical relevance of ANS in human subjects and its long-term safety. Additionally, investigations into the molecular pathways underlying its bioactivity and the development of functional foods or nutraceutical formulations containing *Acacia nilotica* seeds could provide valuable insights for cardiovascular health management and dietary interventions.

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Conflicting Interests

The authors acknowledge that this is not present in the article for the possibility of publishing it.

Ethical Considerations

The ethical considerations for this study were thoroughly reviewed and approved by the Scientific Research Ethics Committee at the Faculty of Home Economics, Menoufia University, Shebin El-Kom, Egypt (Approval # 12-SREC-01-2023).

Authors' Contribution

Yousif Elhassaneen played a key role in designing and developing the study protocol, overseeing the experimental procedures, gathering conceptual data, reviewing and confirming the results and statistical analyses, and supporting the preparation and revision of the manuscript. Esraa S. Abd El-Aty was responsible for conducting the experiments, collecting, organizing, and analyzing the data, gathering relevant information, and drafting the manuscript. Basma A. El-Khateeb helped in formulating the study protocol, supervising the experimental phase, gathering conceptual insights, validating the results, and contributed to writing the manuscript.

Abbreviations

AA, antioxidant activity; Abs, absorbance; Alb, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase activity; ANS, *Acacia nilotica* seeds powder; AST, aspartate aminotransferase activity; BWG, body weight gain; CAT, catalase; Cho, cholesterol; FI, feed intake; FER, feed efficiency ratio; GSH, reduced glutathione; GSH-PX, glutathione peroxidase; HDL-c, High density lipoprotein-cholesterol; LDL-c, low density lipoprotein-cholesterol; MDA, malondialdehyde; ROS, reactive oxygen species; SD, standard deviation; SOD, superoxide dismutase; TGs, triglycerides; TP, total protein.

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