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Research Article

Mineral Content, Antioxidant Properties *in vitro*, Reduction of Inflammation, and Liver Steatosis *in vivo* by Ngaoundal Propolis in Wistar Rats Fed an Atherogenic Diet

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Abstract

Several studies have reported the benefits of Propolis in the treatment of various disorders such as parasitic infections, bacterial infections, wounds, and burns. The overall aim of this study was to evaluate the preventive effects and anti-inflammatory activities of the hydroethanolic extract (EthP) and the fraction powder ≤ 125 µm of Propolis (PP) on atherogenic diet-induced non-alcoholic fatty liver disease. Dry Propolis was finely ground, a first part was macerated in a mixture (30:70 v/v water and ethanol) and a second part was fractionated by sieving with a sieve mesh (≤125 µm). The powder fraction≤ 125µm (PP) and Propolis hydroethanolic extract (EthP) obtained were used to characterize the mineral composition *in vitro* and *in vivo* antioxidant and anti-inflammatory properties. 20 male Wistar rats were divided into 5 groups EthP and PP were administered orally to the rats at the same dose (250 mg/kg bw) and fed simultaneously with an atherogenic diet for 45 days. At the end of the experiment, the lipid profile, transaminase aspartate aminotransferase (AST), and alanine aminotransferase (ALT) in serum, and antioxidants were measured at the organ level (aorta, liver, kidney, and heart). The activities of all parameters were significant ($p < 0.05$). The results of this study show that Propolis had a significantly (*p<0.0001*) lower *in vitro* mineral composition in Iron by 32.56%; in Zinc by 83.21%; in Calcium by 10.82% and in Manganese by 21.40% at the PP level compared to EthP. Antioxidant capacity (DPPH, TAC, and FRAP), which increased with Propolis concentration. High polyphenol content (EthP>PP). Treatment with $EthP_{250}$ and PP_{250} significantly (*p<0.05*) reduced serum ALT by 34.27% and 47.36%, creatinine by 67.36% and 37.5%, TG by 63.91% and by 20.18%, IL-17 expression by 50.25% and 100% respectively. HDL-c levels were significantly increased by 47.7% (*p<0.001*) in serum compared with TN. NO levels increased significantly (*p<0.001*) by 1.38% and 1.63% in the aorta respectively. MDA levels were significantly reduced by 55.12% (*p<0.0001*) and 76.09% (*p<0.05*) in the liver respectively. This study demonstrated the efficacy of Propolis in the management of non-alcoholic hepatic steatosis and its anti-inflammatory capacity.

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1. Introduction

Propolis is a natural substance derived from the harvesting by specialized worker bees [1] of plant resins from leaf buds, stems, or flowers of plants, and is used as protection in the hive [2].

Non-alcoholic hepatic steatosis is defined as an accumulation of fat in the liver in the form of triglycerides in more than 5% of hepatocytes in the absence of other liver disease etiologies or secondary causes of hepatic steatosis [3]. In Africa, prevalence is estimated at 13.48% [4]. In Cameroon, a prevalence of 48.9% has been found among patients with metabolic syndrome [5]. According to the WHO, 80% of the population in developing countries rely on traditional medicine for basic health care [6]. Propolis can be used in several extracted forms, namely hydroethanolic extraction [7-9] or other conventional methods. However, these techniques have their limitations, notably the use of solvents, which can be harmful to the environment and can also be expensive. Faced with this situation, another new extraction technique has been developed. This is the Pulverization and Controlled Differential Screening process (CDSp). This is a dry extraction technique for extracting bioactive compounds from a body according to their granulometric size, developed by Baudelaire [10]. However, to the best of our knowledge, no studies have been conducted on the anti-inflammatory activities of the hydroethanolic extract and the CDSp powder fraction ≤ 125 μm of Propolis on non-alcoholic hepatic steatosis. This raised the research question: what are the effects of (EthP) and (PP) on hepatic steatosis? To answer this question, our general objective is to evaluate the antioxidant and anti-inflammatory activities of the hydroethanolic extract and the CDSp powder fraction ≤ 125 μm of Propolis on atherogenic diet-induced non-alcoholic hepatic steatosis in Wistar rats. More specifically, we will determine the mineral composition, *in vitro* and *in vivo* antioxidant and anti-inflammatory activities of the hydroethanolic extract (EthP), and powder fraction ≤ 125 μm of Propolis (PP) on atherogenic diet-induced steatosis in Wistar rats. anti-inflammatory activities of the hydroethanolic extract and on atherogenic diet-induced steatosis in Wistar rats.

2. Materials and Methods

2.1. Materials

2.1.1. Plant Material

Propolis was obtained in the Ngaoundal (Adamawa Region, Cameroon) locality in December 2021. It was harvested by hand from the hives and stored at room temperature (28 ± 1 ℃) in the dark.

2.1.2. Experimental Animals

The animals used in this study were male rats of Wistar strain, 8-12 weeks old and weighing between 180 and 200g. They were obtained from the LABBAN animal facility (Laboratory of Biophysics, Food Biochemistry and Nutrition of ENSAI, University of Ngaoundere, Cameroon). They were kept under favorable rearing conditions, with temperature (25 ± 3 ℃), humidity between 60 -70%, and a photoperiod of 12 hours during the day and 12 hours at night. The animals had free access to standard feed and water [11].

2.2. Methods

2.2.1. Fractionation and Preparation of Propolis Hydroethanolic Extract

i. Propolis fractionation

Propolis was harvested, dried, and cleaned of all debris. It was then crushed with a mortar to obtain a pre-powder. The pre-powder was ground in a grinder (Biobase Disintegrator MPD-102) to obtain a fine powder. Fractionation was carried out using the Controlled Differential Pulverization and Sieving (CDPS) process described by Deli et al. [12] using an Endecotts sieve shaker (Minnor 1332-06). This method involved depositing a given mass of the powder at the top of a column of sieves with decreasing mesh size, to which a vertical vibratory motion would be applied for a given time. In our study, 370 g of Propolis powder, divided into batches of 30 g, were deposited at the top of a column of sieves with decreasing mesh sizes (250 μm, 200 μm, and 125 μm), placed on a platform directly connected to the sieve shaker motor shaft. The sieve shaker was directly subjected to a continuous vertical vibratory motion for 15 minutes. After passing through the mesh, the powders are retained according to their particle size. The powder fraction used for our study was from the sieve with a mesh diameter of less than 125 μm. Of this fraction, the mass obtained after weighing on a balance (Biobase Biodustry Shandong) was 16.23g. The yield was 7.77%, according to the following formula (1):

$$R = \frac{M}{M0} \text{ x } 100 \tag{1}$$

R: Yield; M: Mass in grams of the fraction obtained; M0: Mass in grams of the Propolis powder used.

ii. Preparation of hydroethanolic extract of Propolis (EthP)

EthP was prepared as described by Djongra et al. [13]. After clearing the crude Propolis of debris, 298 g of crude Propolis were cut into small pieces. In a clean container, these pieces were mixed with 1400 mL of a hydroethanolic mixture (30: 70 v/v water and ethanol), then stirred 3 times a day for 72 hours at 37 °C. The macerate was filtered through Whatman N °4 filter paper. After filtration, we obtained 900 mL, which was evaporated under study (40 °C), yielding 19.91g of concentrated Propolis hydroethanolic extract in the form of a brown oily paste, stored at +4 °C. The extract yield was 6.6%.

2.2.2. Total Phenolics, Flavonoids, and Condensed Alkaloids Contents

The spectrophotometric technique used as the Folin-Ciocalteu reagent described by Wafa et al. [14] was used for the determination of total polyphenols. The results were expressed in mg gallic acid equivalent per gram of dry weight (GAE/g DW).

Total flavonoids were evaluated by the colorimetric method described by Dewanto et al. [15]. The results were expressed in mg quercetin equivalent per gram of dry weight (mg QE/g DW).

The condensed alkaloid content of each particle size class was assessed according to the method described by Sun et al. [16] with slight modifications. The condensed alkaloid content was expressed as gram catechin equivalent per 100 grams of dry weight (g CE/100 g DW).

2.2.3. Minerals-Elements Content

Minerals elements (Manganese, Iron, Selenium, Zinc, Copper, and Vitamin C) content of hydroethanolic extract and fraction powder $\leq 125\mu m$ of Propolis powder were determined by employing the AOAC [17].

2.2.4. *In-vitro* Antioxidant Activity of Hydroethanolic Extract and Fraction Powder $\leq 125\mu m$ of Propolis

i. DPPH radical-scavenging activity assay

The DPPH radical scavenging activity of the hydroethanolic extract and the powder fraction ≤ 125 µm of Propolis have been measured according to the method of Zhang and Hamauzu [18] with slight modifications. IC_{50} values were obtained by plotting the DPPH scavenging effect (%) as a function of concentration followed by extrapolation (2) and (3).

$$Y(EthP) = 29.502 \ln(x) + 28.268; \ R^2 = 0.9662 \quad (2)$$

$$Y(PP) = 25.963 \ln(x) - 7.6658; \ R^2 = 0.803 \quad (3)$$

ii. Total antioxidant capacity (TAC)

The total antioxidant capacity of hydroethanolic extract and the powder fraction ≤ 125 µm of Propolis of different samples was evaluated by the phosphomolybdenum method of Prieto et al. [19]. Total antioxidant capacity was expressed as micrograms equivalents of ascorbic acid per gram of dry weight (µgAA/g DW).

iii. Ferric Reducing Ability (FRAP) Test

The FRAP assay was carried out according to Benzie and Strain with some modifications [20]. The FRAP assay was expressed as micrograms equivalents of ascorbic acid per gram of dry weight (µgAA/g DW).

2.2.5. Induction of Non-Alcoholic Fatty Liver Disease and Treatment of Animals

After one week of acclimatization, twenty (20) male rats were divided into 5 groups of 4 rats, and the normal diet and atherogenic diet were formulated according to Tsague et al. [21] for 45 days as follows:

1. Normal control group: received distilled water (1 mL/100 g bw/day, *per os*) and fed a normal diet;
2. Negative control group: received distilled water (1 mL/100 g bw/day, *per os*) and was fed an atherogenic diet;
3. Positive control group: Atorvastatin (10 mg/kg bw/day, *per os*) and was fed an atherogenic diet;
4. Test group 1: received a hydroethanolic extract of Propolis (EthP) (250mg/kg bw/day, *per os*) and was fed an atherogenic diet;
5. Test group 2: received Propolis fraction ≤ 125 µm (PP) (250 mg/kg bw/day, *per os*) and was fed an atherogenic diet.

During 45 days of experimentation, the body weight of each rat was recorded every 5 days, and the food intake was recorded weekly, or even according to the formula (4).

$$consumption \frac{water}{food} = \frac{Average\ consumption \frac{water}{food}}{Average\ lot\ weight} \ x\ 100 \quad (4)$$

At the end of the treatment, the animals were fasted for 12 hours and finally sacrificed under anesthesia with Ketamine/Valium (0.2 mL/0.1 mL per 100g bw in IP). Arterial blood was collected in dry tubes, by sectioning the jugular artery, then centrifuged at 2500 rpm for 15 min. Serum was collected, aliquoted, and stored at -20 °C for analysis of biochemical parameters of interest. The organs (aorta, heart, liver, and kidneys) were carefully isolated, then washed in 0.9% NaCl solution, wrung out, and weighed. A 0.6 g fraction of each organ was removed and triturated in 2 mL Phosphate buffer (0.1M, pH 7.2) for the liver and kidneys, and in 2 mL Mac Ewen buffer (pH 7.4) for the aorta and heart. The remaining liver was placed in 10% formalin for histological sections. Homogenates were centrifuged at 3500 rpm for 15 minutes. The supernatant was collected and stored at -20 °C for analysis (Tsague et al. [21]).

Composition	Normal diet (%)	Atherogenic diet (%)
Protein: fish powder	12	10
Carbohydrates: 50% pulped corn flour + 50% wheat flour	71	61
Sugar: table sugar	05	05
Fatty acid: lard	05	16
Salts: cooking salts 3%+calcium 1%	04	04
Fiber: cellulose	02	01
Cholesterol: cooked egg yolk	-	01
vitamins	01	02
Total weight (g)	100	100

2.2.6. Evaluation of Biochemical Parameters

Biochemical analyses were performed using an APPEL PD-303S spectrophotometer.

2.2.7. Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST), and Creatinine Assay

i. Determination of alanine aminotransferase (ALT) and aspartate aminotransferase (AST)

The determination of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) was performed using SBio reagents, Biosciences PTELtd. Malaga, Spain, Ref.90770075.

The activity of ALT and AST was described by Wallnofer et al. [23]. Activity values are expressed in IU/L.

ii. Creatinine Assay

Creatinine was determined by the enzymatic colorimetric method using the kit: MonlabTest, Ref: MO-165082, and the optical density reading was taken at a wavelength of 492 nm. Results are expressed in mg/dL [24].

2.2.8. Assessment of Lipid Profile Parameters, Inflammation Marker Assay: Interleukin-17, Measurement of Oxidative Stress Marker Activity in the Aorta, Heart, Liver, and Kidney

i. Total cholesterol assay technique

Total cholesterol was determined by the enzymatic colorimetric method using the Human kit Ref: 10017 and read at a wavelength of 593nm. Results are expressed in mg/dL [25].

ii. Triglyceride assay technique

Triglycerides were determined by the enzymatic colorimetric method using the SBio kit, Biosciences Ref: 90810075. Optical density was read at a wavelength of 546 nm. Results are expressed in mg/dL [26].

iii. HDL-c assay technique

HDL-cholesterol was determined by the enzymatic colorimetric method using the Human kit Ref:10084. Optical density was read at a wavelength of 593 nm. Results are expressed in mg/dL [27].

iv. Determination of LDL-cholesterol (LDL-c)

LDL-cholesterol was determined using Friedwald's formula.

[LDL-c] (mg/dL) = [Total Cholesterol] - [HDL-c] -[TG]/5 [27].

v. Inflammation Marker Assay: Interleukin-17

The IL-17 assay was performed using a *RayBiotech* ELISA kit, and optical densities (O. D.) were read using the HumanReader HS automated system [21].

vi. Measurement of tissue-reduced glutathione, superoxide dismutase (SOD); malondialdehyde (MDA), and nitric oxide (NO) A activities

Reduced glutathione activity was measured by the method of Ellman [28]. Absorbance is read at a wavelength of 412 nm. Tissue glutathione concentration in μmol/g of the organ.

Superoxide dismutase activity was measured by the method of Misra and Fridovish [29]. Absorbance is read at a wavelength of 480 nm. SOD activity is determined in units of SOD/g of the organ.

Malondialdehyde activity was measured using the method of Wilbur et al. [30]. Absorbance is read at a wavelength of 530 nm. Malondialdehyde concentration in tissue in μmol/g of the organ.

Nitric oxide activity was measured using the method of Grand et al. [31]. Absorbance is read at a wavelength of 570 nm. Tissue nitric oxide concentration in mmol/g of organ.

2.2.9. Histological Section of Liver

Stained liver tissue sections were carefully examined under

an Olympus CH_2 photonic microscope in the histology laboratory of the Yaounde University Hospital. Tissue sections from all groups were examined for fatty deposits compared with the normal control group. Photomicrographs of selected slides from the different groups were taken under $10\times$ and $40\times$ magnification using an Olympus 101 integrated digital camera.

2.2.10. Statistical Analysis

Results were expressed as mean ± standard error of the mean. Analysis of variance (ANOVA) and Tukey's multiple comparison tests for significant differences were performed using GraphPad Prism 8.0.1.244 software. Graphs were plotted using Microsoft Excel 2016 software. Statistical significance is defined for *p<0.05*.

3. Results

The study aimed to evaluate the powerful antioxidant and anti-inflammatory of Propolis.

3.1. Minerals-Elements Content

Vitamin C, Iron, Manganese, Zinc, Copper, Selenium, and Calcium were determined in the EthP and PP, as demonstrated in Table 2.

Table 2. Composition in some minerals of hydroethanolic extract of Propolis (EthP) and powder fraction ≤ 125μm (PP).

Constituents (g/100g DW)	Hydroethanolic extract EthP	Powder fraction PP
Dry matter content	80.44 ±0.42	92.62 ±5.36****
Ash content	6.79 ±0.33	7.87 ±0.92***
Moisture	19.56 ±0.42	7.37 ±5.36****
Vitamin C	79.53 ±2.86	56.93 ±4.13****
Copper	4.36 ±0.43	4.66 ±1.1
Zinc	10.56 ±0.26	12.69 ±0.25***
Selenium	0.023 ±0.023	0.026 ±0.002
Manganese	2.53 ±0.33	11.85 ±0.04****
Iron	12.11 ±0.41	37.19 ±0.41****
Calcium	213.4 ±12.56	253.10 ±3.3****

Values are expressed as means±SEM; n=3; *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001 significant difference from EthP.
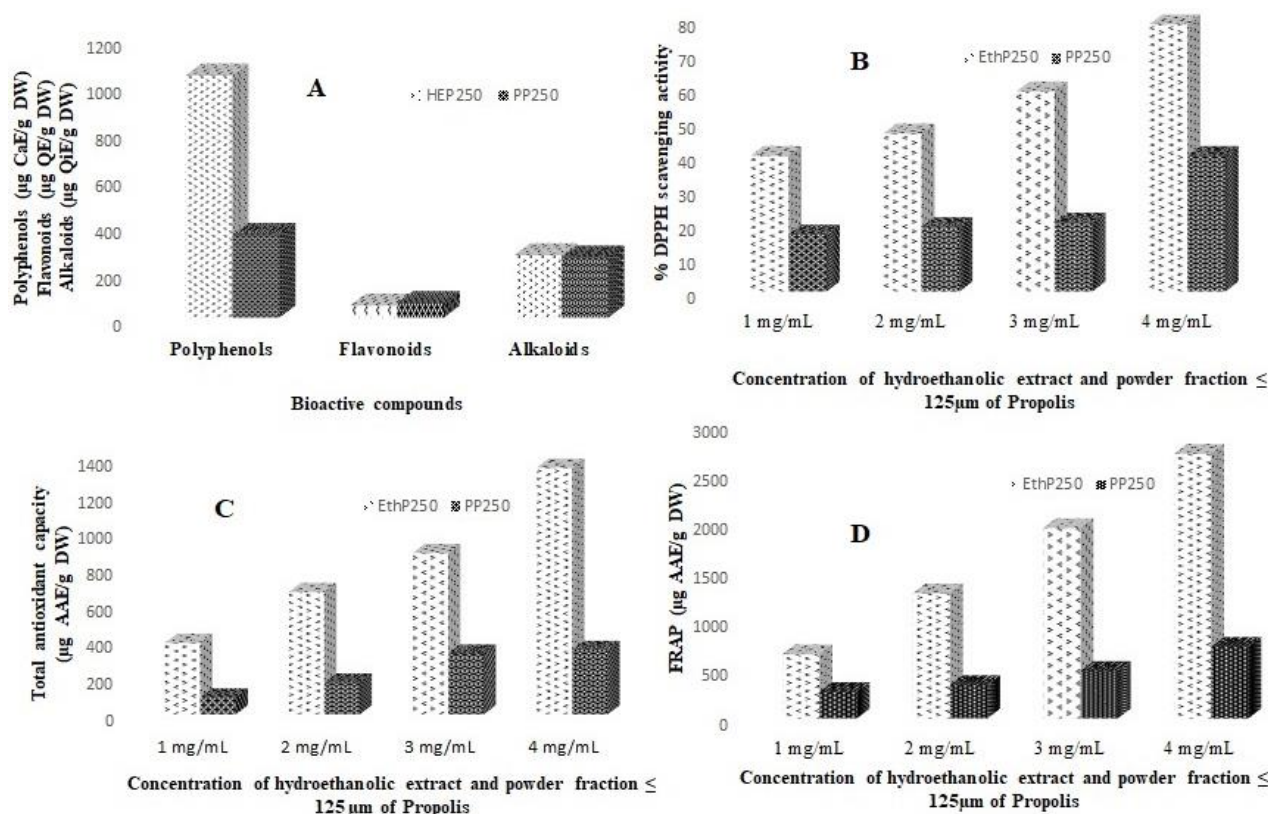
EthP: Hydroethanolic extract of Propolis; PP: Powder fraction ≤125μm of Propolis; ≤125μm: Powdery fractions with a diameter inferior at 125 μm

Ash content was significantly (*P<0.0001*) higher in PP (86.27%) than in EthP. Moisture content was significantly (*P=0.001*) decreased in PP (37.67%) compared to EthP. Zinc, Manganese, Iron, and Calcium were significantly (*P<0.05*) increased in PP 83.21%, 21.35%, 32.56%, and 84.31% respectively compared to EthP. While Vitamin C was significantly decreased (*P=0.0001*) in PP (71.58%) compared to EthP.

3.2. *In-vitro* Antioxidant Activity of Hydroethanolic Extract and Fraction Powder ≤ 125μm of Propolis

Figure 1 shows the bioactive compounds: polyphenols, flavonoids, and alkaloids (A), and antioxidant activities: DPPH scavenging activity (B), Total Antioxidant Capacity (C), and Ferric Reducing activity Potential (D) of EthP and PP.

Figure 1. Bioactive compounds (A) and antioxidant activities: Percentage of DPPH scavenging activity (B), Total antioxidant capacity (C), and Ferric Reducing activity Potential (D) of hydroethanolic extract and powder fraction ≤125μm of Propolis at different concentrations.

μg: Microgramme; AAE: Ascorbic Acid Equivalents; CaE: Catechin Equivalent; g: Gramme; DW: Dry Weight; EQ: Quercetin Equivalent; QiE: Quinine Equivalent; EthP: Hydroethanolic extract of Propolis; PP: Propolis powder fraction ≤ 125μm; ≤125μm: Powdery fractions with a diameter inferior at 125 μm

Figure 1A showed that polyphenol content was significantly ($p<0.05$) high in EthP (1037.80 ± 16.99 μg CaE/ g DW) compared to PP (354 ± 5.08 (μg CaE/g DW)); flavonoid content was significantly ($p<0.05$) higher in PP (62.25 ± 3.61 (μg QE/g DW)) than in EthP (51.20 ± 2.68 (μg QE/g DW)). However, the alkaloid content in EthP (267.3 ± 13.43 μg QiE/ g DW) was significantly ($p<0.05$) lower than in PP (257.05 ± 4.23 μg QiE/ g DW).

The DPPH radical scavenging activity of the EthP and PP is shown in Figure 1B. After observation, it was noted that the different samples trapped this synthetic radical in a concentration-dependent manner (activity increases in parallel with increasing extract or powder concentrations). The PP with a CI_{50} of 93.90mg/mL exhibited the best activity.

$$Y(EthP) = 29.502 \ln(x) + 28.268; R^2 = 0.9662\ IC_{50} = 143.68 \text{ mg/mL} \quad (5)$$

$$Y(PP) = 25.963 \ln(x) - 7.6658; R^2 = 0.803\ IC_{50} = 93.90 \text{ mg/mL} \quad (6)$$

3.3. Effect of Hydroethanolic Extract and Fraction Powder ≤ 125 μm of Propolis on Weight Change

The hydroethanolic extract and the fraction power ≤ 125 μm of Propolis were administered to the rats during the 45 days of experimentation at the end of which the weight change was recorded and is mentioned in Figure 1. The normal diet group of rats increased body weight up to the 45th day of experimentation. The negative control group, which was fed the atherogenic diet, saw weight increase significantly ($p<0.01$) up to day 10, followed by a significant ($p<0.001$) decrease up to day 45. On day 5, the $EthP_{250}$ and PP_{250} groups significantly ($p<0.05$) increased weight by 60.97% and 61.24% respectively compared with the normal control group.

However, from days 15 and 20 onwards, there was a significant ($p<0.001$) decrease in body weight for $EthP_{250}$ and PP_{250} followed by a significant ($p<0.001$) increase in weight on day 25, before a significant ($p<0.0001$) decrease in weight on day 45 respectively compared with the normal control. At day 45, the $EthP_{250}$ and PP_{250} groups showed a small significant decrease ($p<0.001$) compared with the negative control group.

Table 3. *Effect of hydroethanolic extract and powder fraction ≤ 125 µm of Propolis on transaminase activities, creatinine level, and the lipidic profile.*

	ND	AD	AD Atorvastatin (10mg/kg)	EthP$_{250}$	PP$_{250}$
ALT (U/L)	34.15 ±2,59	64.48 ±0.14[***]	38.98 ±6.55[##]	22.10 ±2.52[####]	30.54 ±2.33[####]
AST (U/L)	34.54 ±0.11	85.75 ±0.21[****]	62.24 ±4.53[**#]	46.3 ±1.26[###]	68.14 ±5.51[***α]
AST/ALT	1.01	1.32	1.59	2.09	2.23
Creatinine (mg/dL)	1.28 ±0.1	1.9 ±0.04[*]	1.68 ±0.16	0.6 ±0.07[**###]	1.6 ±0.14[ααα]
TC (mg/dL)	115.3 ±1.8	161.2 ±4.5[***]	143.05[***##]	119.6 ±2.6[###]	118.8 ±2.5[###]
TG (mg/dL)	91.5 ±1.4	98.1 ±0.9[*]	85.2 ±1.7[*###]	62.7 ±1.34[***###]	19.8 ±0.67[***###ααα]
LDL-C (mg/dL)	42.7 ±5.9	104.8 ±9.4[*]	36.96 ±9.15[#]	29.47 ±11.46[##]	27.3 ±17.62[##]
HDL-C (mg/dL)	53.23 ±1.19	39.42 ±1.3[**]	84.65 ±4.16[***###]	82.63 ±1.36[****####]	95.56 ±3.01[****####αα]
CT/HDL-C	2.16	4.08	1.68	1.44	1.24
(%) Protection	-	-	58.82	64.70	69.60

ND: Normal diet; AD: Atherogenic diet; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; TC: Total cholesterol; TG: Triglycerides; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; EthP$_{250}$: Hydroethanolic extract of Propolis at the dose 250mg/kg bw; PP$_{250}$: Propolis powder fraction ≤ 125µm at the dose 250mg/kg bw; ≤12µm: Powdery fractions with a diameter inferior at 125 µm.

Values are expressed as means ± SEM; n=3; *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001 significant difference from the control group; [≠]p<0.05; [##]p<0.01; [###]p<0.001; [###]p<0.0001 significant difference from the negative control group; [α]p<0.05; [αα]p<0.01; [ααα]p<0.001; [αααα]p<0.0001 significant difference from the EthP group.

3.4. Effect of Propolis Hydroethanolic Extract and Fraction Powder ≤ 125 µm on the Liver and the Kidney Function Markers

Effect on transaminase (ALT, and AST) activity and creatinine levels

Table 3 shows the effect of treatment with EthP$_{250}$ and PP$_{250}$ on ALT, AST, and creatinine levels in rats after 45 days of experimentation. It was found that feeding the atherogenic diet induced a significant increase in ALT, AST, and creatinine activities of 52.96% (*P<0.001*), 40.27% (*P<0.0001*), and 67.36% (*P<0.05*) respectively in the TN group compared with the normal control group. Treatment of animals with EthP$_{250}$ resulted in a significant decrease in ALT, AST, and creatinine activities of 34.27% (*P<0.0001*), 53.99% (*P<0.001*), and 31.57% (*P<0.05*) respectively compared to the TN group. Whereas PP$_{250}$ only resulted in a 47.33% (*P<0.0001*) decrease in AST activity compared with the TN group. However, EthP$_{250}$ significantly decreased serum AST and creatinine levels by 67.94% (*P<0.05*) and 37.5% (*P<0.001*) versus the PP$_{250}$ group.

3.5. Effect of Hydroethanolic Extract and Propolis Fraction Powder ≤ 125 µm on Lipid Profile

Feeding the rats an atherogenic diet produced the results on the lipid profile of the animals shown in Table 3.

This table shows that the serum total cholesterol concentration of animals in the TN group increased significantly (*p<0.001*) by 71.52% compared with the normal control. On the other hand, treatment with EthP$_{250}$, and PP$_{250}$ significantly (*p<0.001*) lowered cholesterol levels by 74.19% and 73.69% respectively, compared with the TN group.

After 45 days of experimentation, rats on the atherogenic diet (TN) showed a significant (*p<0.05*) 93.27% increase in TG levels compared with the normal control group. The administration of EthP$_{250}$, and PP$_{250}$ as preventive treatment resulted in a significant (*p<0.001*) decrease in serum triglyceride levels of 63.91% and 20.18% respectively compared to the TN group. However, the PP$_{250}$ significantly (*p<0.001*) reduced triglyceride levels by 31.57% compared with the group receiving the EthP$_{250}$ at the same dose.

At the end of the experiment, LDL-c levels increased significantly (*p<0.05*) by 40.74% in the TN group compared with the normal control. Treatment with EthP$_{250}$ and PP$_{250}$

significantly ($p<0.01$) reduced LDL-c concentration by 28.12% and 26.04% respectively, compared with the TN group.

The HDL-c results presented in Table 4 show a significant ($p<0.01$) decrease in HDL-c levels of 74.05% in the TN group compared with the normal control. There was a significant increase ($p<0.0001$) of 47.7% and 41.25% when EthP$_{250}$ and PP$_{250}$ were administered compared with the TN group.

However, treatment with PP$_{250}$ showed a significant ($p<0.01$) 86.46% increase in HDL-c concentration, compared with treatment with EthP$_{250}$, Table 3 showed that rats fed an atherogenic diet for 45 days were at risk of developing atherosclerosis and consequently cardiovascular disease. Propolis reduced this risk by 64.70% and 69.60% respectively with EthP$_{250}$ and PP$_{250}$.

***Table 4.** Effect of hydroethanolic extract and powder fraction ≤ 125 µm of Propolis on some Biomarkers.*

		ND	AD	AD Atorvastatin (10mg/kg)	EthP$_{250}$	PP$_{250}$
NO (mmol/g of organ)	Liver	1.21 ±0.24	0.44 ±0.12	1.51 ±0.15$^{\#}$	1.21 ±0.13	1.16 ±0.14
	Kidney	1.05 ±0.15	0.7 ±0.19	0.55 ±0.08	1.31 ±0.13	1.59 ±0.28
	Heart	0.33 ±0.03	0.22 ±0.02	0.37 ±0.03	0.67 ±0.19	0.33 ±0.08
	Aorta	0.42 ±0.008	0.01 ±0.002**	0.58 ±0.12$^{\#\#\#\#}$	0.72 ±0.075$^{\#\#\#\#}$	0.61 ±0.03$^{*\#\#\#\#}$
Glutathione (µmol/g of organ)	Liver	34.87 ±2.25	63.07 ±5.87*	32.48 ±2.91$^{\#}$	52.57 ±8.88	35.85 ±3.45
	Kidney	39.37 ±3.5	52.61 ±6.1	33.46 ±0.94$^{\#\#}$	56.83 ±1.37**	58.85 ±2.14**
	Heart	98.5 ±0.5	138.1 ±4.7**	101.3 ±8.5$^{\#\#}$	97.71 ±5.43$^{\#\#}$	126.6 ±7.74$^{\beta}$
	Aorta	31.5 ±3.6	15.69 ±0.49	45.28 ±2.51	46.81 ±0.28	31 ±0.14
SOD (UI/g of organ)	Liver	56.3 ±8.7	139.1 ±11.35***	86.19 ±8.7$^{\#}$	96.22 ±3.3	88.77 ±5.79
	Kidney	46.4 ±3.3	209.1 ±41.47***	66.32 ±3.3	109.1 ±5.6	129.2 ±1.04
	Heart	69.65 ±17.23	182.5 ±14.4	63.02 ±3.31***	86.02 ±3.21$^{\#\#}$	82.92 ±11.96$^{\#\#}$
	Aorta	74.63 ±14.36	238.6 ±5.85****	99.38 ±0.07$^{\#\#\#\#}$	69.65 ±11.49$^{\#\#\#\#}$	34.33 ±3.16$^{\#\#\#\#}$
MDA (µmol/g of organ)	Liver	1.38 ±0.13	2.05 ±0.001	1.49 ±0.12$^{\#\#}$	1.13 ±0.06$^{\#\#\#\#}$	1.56 ±0.08$^{\#\beta}$
	Kidney	1.004 ±0.08	2.009 ±0.02****	1.15 ±0.02$^{\#\#\#\#}$	1.15 ±0.07$^{\#\#\#\#}$	1.28 ±0.07$^{\#\#\#\#}$
	Heart	1.02 ±0.09	1.85 ±0.06****	1.06 ±0.001	1.21 ±0.03$^{\#\#\#}$	1.23 ±0.04$^{\#\#\#}$
	Aorta	1.53 ±0.04	3.07 ±0.81	1.47 ±0.01$^{\#}$	1.43 ±0.03$^{\#}$	1.49 ±0.001$^{\#}$

ND: Normal diet; AD: Atherogenic diet; NO: Nitrite oxide; SOD: Superoxide dismutase; MDA: Malondialdehyde; EthP$_{250}$: Hydroethanolic extract of Propolis at the dose 250mg/kg bw; PP$_{250}$: Propolis powder fraction ≤ 125µm at the dose 250mg/kg bw; ≤12µm: Powdery fractions with a diameter inferior at 125 µm.

Values are expressed as means ± SEM; n=3; *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001 significant difference from the control group; $^{\#}$p<0.05; $^{\#\#}$p<0.01; $^{\#\#\#}$p<0.001; $^{\#\#\#}$p<0.0001 significant difference from the negative control group; $^{\alpha}$p<0.05; $^{\alpha\alpha}$p<0.01; $^{\alpha\alpha\alpha}$p<0.001; $^{\alpha\alpha\alpha\alpha}$p<0.0001 significant difference from the EthP group.

3.6. Effect of Hydroethanolic Extract and Fraction Powder ≤ 125µm of Propolis on Interleukin-17

IL-17 expression levels are shown in Figure 2. It can be seen that the TN group showed a significant increase ($P<0.0001$) in IL-17 expression levels of 35.5% compared

with the normal control. Treatment with EthP$_{250}$ and PP$_{250}$ significantly ($p<0.001$) reduced IL-17 expression levels by 50.25% and 100% respectively compared with the TN group. Results showed that serum IL-17 expression was significantly reduced ($p<0.001$) in the PP$_{250}$ group by 100% compared with the EthP$_{250}$ group.

Figure 2. *Variation of body weight.*

EthP$_{250}$: Hydroethanolic extract of Propolis at the dose of 250mg/kg bw; PP$_{250}$: Propolis powder fraction $\leq 125\mu m$ at the dose of 250mg/kg bw; $\leq 12\mu m$: Powdery fractions with a diameter inferior at 125 µm.
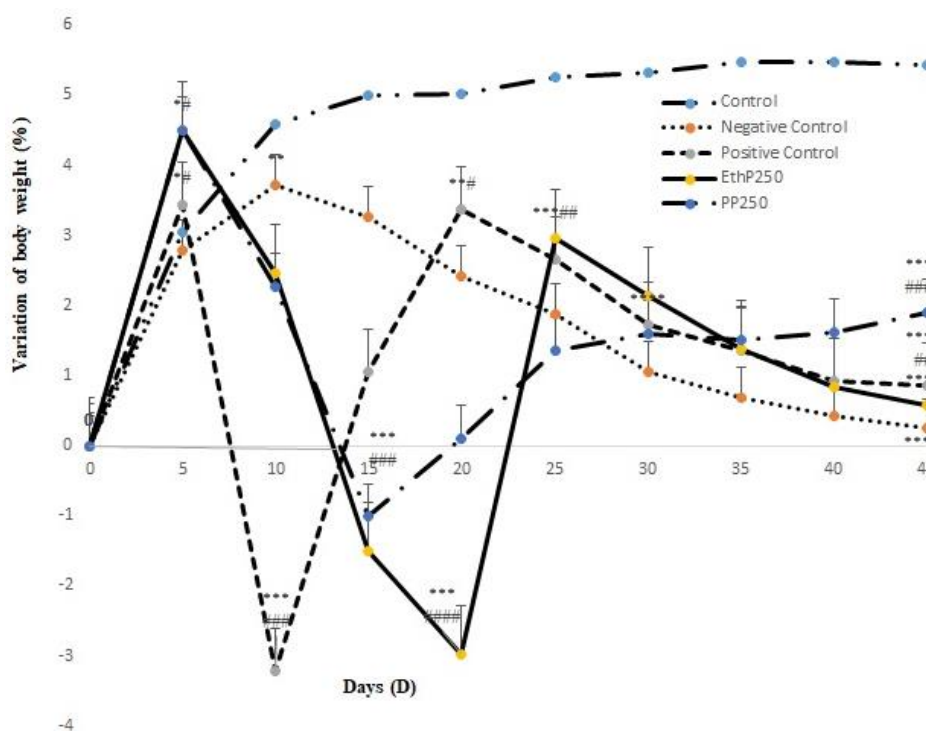Values are expressed as means $\pm$ SEM; n=3; *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001 significant difference from the control group; $^\sharp$p<0.05; $^{\sharp\sharp}$p<0.01; $^{\sharp\sharp\sharp}$p<0.001; $^{\sharp\sharp\sharp}$p<0.0001 significant difference from the negative control group.

Effects of EthP$_{250}$ and PP$_{250}$ on markers of oxidative stress in preventive treatment of cardiovascular disease. Propolis reduced this risk by 64.70% and 69.60% respectively with EthP$_{250}$ and PP$_{250}$.

3.7. Effect of Hydroethanolic Extract and Fraction Powder ≤ 125μm of Propolis on Some Biomarkers

At the end of the experiment, the administration of the atherogenic diet had an impact on markers of oxidative stress. The data received are shown in Table 4.

Table 3 shows that aortic nitrite levels in the TN group decreased significantly ($p<0.01$) by 2.38% compared with the normal control group. On the other hand, when EthP$_{250}$ and PP$_{250}$ were administered, nitrite levels in the aorta increased significantly ($p<0.0001$) by 1.38% and 1.63% respectively, compared with the TN group.

Tissue glutathione levels rose significantly ($p<0.05$) by 55.28% in the liver and 71.32% in the heart ($p<0.01$) respectively, compared with the normal control group (Table 4). Only EthP$_{250}$ caused a significant ($p<0.01$) 70.75% drop in glutathione levels compared with the negative control. Prop-

olis powder, however, significantly increased ($p<0.05$) tissue glutathione levels by 77.18% compared with Propolis hydroethanolic extract.

According to Table 4, SOD activity increased significantly ($p<0.001$) by 40.47% in the liver, 22.19% in the kidney, and 31.27% ($p<0.0001$) in the aorta, respectively in TN compared with the normal control. Rats given the EthP$_{250}$ and PP$_{250}$ of Propolis showed a significant ($p<0.01$) decrease of 47.13% and 45.43% respectively in the heart; 29.19% and 14.38% ($p<0.0001$) respectively in the aorta compared with TN.

Malondialdehyde levels assessed in experimental rats showed a significant increase ($p<0.0001$) in TN of 49.97% and 55.13% respectively in the kidney and heart compared with the normal control (Table 4). Compared with NT, rats treated with EthP$_{250}$ and PP$_{250}$ showed a significant decrease in MDA levels in the liver by 55.12% ($p<0.0001$) and 76.09% ($p<0.05$) respectively; in the kidney by 57.24% ($p<0.0001$) and 63.71% ($p<0.0001$) respectively. In the heart, these values were 65.40% ($p<0.001$) and 66.48% ($p<0.001$) respectively; in the aorta, 46.57% ($p<0.05$) and 48.53% ($p<0.05$).

However, the administration of PP$_{250}$ significantly ($p<0.05$) increased MDA levels by 72.43% compared with EthP$_{250}$.
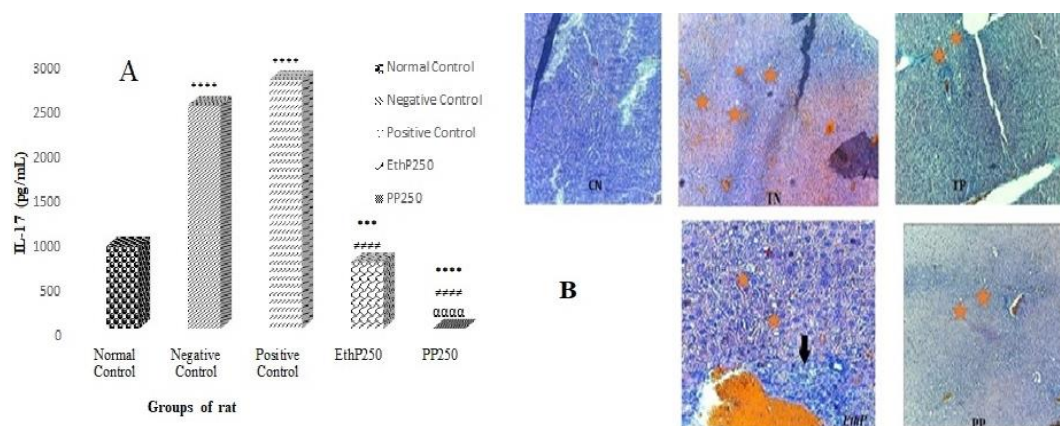
Figure 3. Effect of hydroethanolic extract and powder fraction ≤ 125µm of Propolis on the Interleukin-17, determined by ELISA assays (A), Histological section of the liver, and effects of Propolis on lipid accumulation (B).

Values are expressed as means ±SEM; n=4;***p<0,001; ****p<0.0001 significant difference from the control group; [###]p<0.0001 significant difference from the negative control group; [αααα]p<0.0001 significant difference from the EthP group. ####p<0.00: significant differences from negative group; [αααα]p<0.001 significant differences from EthP treatment group EthP$_{250}$: Hydroethanolic extract of Propolis at the dose of 250mg/kg bw; PP$_{250}$: Propolis powder fraction ≤ 125µm at the dose of 250mg/kg bw; IL-17: Interleukin 17; Negative Control: Moderate steatosis x 10; Positive Control: mild steatosis and mild inflammation x10; EthP250: mild steatosis x 40; PP250: mild steatosis x 10.

Presence of hepatic steatosis ⭐

Presence of inflammation ➡

4. Discussion

This study evaluated the content of certain mineral elements (Vitamin C, Iron, Manganese, Zinc, Copper, Selenium, and Calcium), determined antioxidant activity *in vitro* (DPPH, TAC, and FRAP), demonstrated the reduction in inflammation and hepatic steatosis *in vivo* of Propolis hydroethanolic extract (EthP) and powder fraction ≤ 125 µm (PP) in Wistar rats fed an atherogenic diet.

4.1. Minerals-Elements Content

Fractionation and sieving of Propolis to a powder fraction≤125µm resulted in a significant increase in dry matter, center content, Zinc, Magnesium, Iron, and Calcium. This may be explained by the fact that sieving favors the concentration of certain bioactive compounds. The increase in ash content with the reduction in particle size could be explained by the fact that during sieving the ash, which was less heavy, was found in the smaller particle size classes [32].

4.2. *In-vitro* Antioxidant Activity of Hydroethanolic Extract and Fraction Powder ≤ 125µm of Propolis

EthP showed better activity than PP, which could be explained by the fact that the polyphenols present in EthP would have a high polarity, as the water-ethanol mixture would favor polar compounds. Similar results were reported by Tsague et al. [22].

The DPPH radical scavenging activities of the EthP and PP are shown in Figure 1B. After observation, it was noted that the different samples trapped this synthetic radical in a concentration-dependent manner (activity increases in parallel with increasing extract or powder concentrations). The PP with a CI$_{50}$ of 93.90mg/mL exhibited the best activity.

Taking a look at Figure 1C for data on the total antioxidant capacity of the PP and EthP, these results confirm previous trends observed through DPPH radical scavenging and iron reduction activities, which showed that the antioxidant potential of our extracts was concentration-dependent. However, the best activity here was shown by the PP, in contrast to the result observed for FRAP potential.

Figure 1D shows the ferric iron reduction capacity of the EthP and PP. These data show that, as in the case of DPPH radical scavenging, the iron reduction potential increased positively with increasing hydroethanolic extract and powder concentration. The EthP exhibited the best activity.

4.3. Effect of Hydroethanolic Extract and Fraction Powder ≤ 125 µm of Propolis on Weight Change

In this study, rats subjected to the atherogenic diet lost weight throughout the experiment. This observed weight loss is thought to be due to the secretion of leptin [33]. Indeed, leptin is a hormone secreted mainly by adipose tissue and acts at the level of the hypothalamus to stimulate the sensation of satiety and stop food intake. This result is in line with those of Tsague et al. [22], who found that this atherogenic diet reduced the weight of the animals simultaneously with food

intake. Feeding with EthP$_{250}$ and PP$_{250}$ significantly improved this weight loss compared with the TN group. These results suggest that EthP$_{250}$ and PP$_{250}$ may have an appetite-stimulating effect on weight gain. At the end of the experiment, PP$_{250}$ showed greater activity than Eth$_{250}$, proving that Pulverization and Controlled Differential Screening process concentrate bioactive compounds in addition to polyphenols. Which stimulated appetite in rats.

4.4. Effect of Propolis Hydroethanolic Extract and Fraction Powder ≤ 125 µm on the Liver and the Kidney Function Markers

Effect on transaminase (ALT, and AST) activity and creatinine levels

Transaminases are enzymes synthesized by hepatocytes, ensuring the synthesis of amino acids by transferring an amino group from an amino acid to ketoglutarate [34]. Excessive fat accumulation in the liver would have caused oxidative stress and liver inflammation, which would have damaged liver cells and led to their lysis, hence the observed increase [35]. They are found in the cytosol (ALT and AST) and mitochondria (AST) of liver cells. They are the most sensitive biomarkers implicated in liver dysfunction and tissue damage [36]. The enzymatic activity of transaminases is increased in the bloodstream due to increased lysis of liver parenchyma cells [37]. When this decreases, there is restoration of plasma membrane stability as well as protection and regeneration of damaged liver cells [38]. The hepatoprotective effect of Propolis is due to its richness in bioactive compounds such as polyphenols [39] and the presence of certain mineral compounds such as Zinc, Cu, Mn, Iron, Ca, Se, and Vit C. These results are in agreement with work presented by Tsague et al. [21] who demonstrated that the presence of these minerals in powder fraction ≤ 125 µm of *Eribroma oblongum* promoted a decrease in anti-inflammatory expression in Wistar strain rats. For this practical reason, the use of Propolis will be essential in solving the problem of non-alcoholic fatty liver disease [36, 40, 41] showed that polyphenols in aqueous extracts of *Scolymus hispanicus* and *Salvia plebeia* reduced liver damage in non-alcoholic fatty liver disease by improving transaminase levels.

The kidney is the site of the excretion of toxic wastes released by the liver [42]. When these wastes exceed the kidney's filtration capacity, we observe a decrease in glomerular filtration in the kidney and a consequent increase in certain substances such as creatinine in the blood [43]. Creatinine is a metabolite of creatine, which in skeletal muscle is phosphorylated to creatine phosphate, a compound rich in free energy [44]. It is a marker of renal function.

4.5. Effect of Hydroethanolic Extract and Propolis Fraction Powder ≤ 125 µm on Lipid Profile

The liver is the main site of lipid metabolism in the body

[45]. Following a meal, chylomicrons transport triglycerides and dietary cholesterol to store some in tissue reserves and some in the liver [46]. In the liver, these TGs are incorporated into VLDLs, which are responsible for supplying fatty acids to user tissues or depositing them in adipose tissue. The circulating VLDLs, as they supply free fatty acid, become IDLs, which are enriched with cholesterol, forming LDLs responsible for supplying esterified cholesterol to user tissues [46].

An atherogenic diet rich in saturated fatty acids and cholesterol can therefore be the source of TG overload in the liver, with an increase in LDL in the circulation followed by a drop in HDL [46]. Propolis may therefore have acted by reducing the secretion of chylomicrons into the lymph and inhibiting the secretion of pancreatic lipase, a key enzyme in the intestinal absorption of dietary lipids. It could also act by increasing the expression of carnitine palmitoyl transferase receptors, which will increase the beta-oxidation of fatty acids to produce energy and thus reduce fat accumulation in the liver [47]. These results are in line with those of Afsharinasab et al. [48] who showed that the hydroalcoholic extract of *Berberis integerrima* improved the lipid profile. Propolis increased HDL-c levels in rats. HDL-c is responsible for capturing cholesterol and returning it to the liver for excretion. Non-alcoholic fatty liver is particularly prone to death from cardiovascular or liver-related causes, as is the inflammation seen in NAFLD [49].

The lipid profile enables direct confirmation of the diagnosis associated with cardiovascular disease. It can be combined with the calculation of the atherogenic index to determine cardiovascular risk, more specifically intermediate risk [50]. The atherogenic index, also known as the Castelli index, is a lipid ratio used to assess the risk of atherosclerosis and cardiovascular disease [50]. Administration of PP$_{250}$ reduced the risk of atherosclerosis by 93.05%. These results are similar to those of Tsague et al. [22], who showed that administration of *Eribroma oblongum* (100mg/kg bw) in rats reduced cardiovascular risk by 89.09%. Propolis (250mg/kg bw) therefore has an anti-steatosis and anti-atherosclerosis effect.

4.6. Effect of Hydroethanolic Extract and Fraction Powder ≤ 125µm of Propolis on Interleukin-17

IL-17 is a pro-inflammatory cytokine that is ubiquitously expressed in liver cells, which explains its involvement in liver damage [51]. Atherogenic feeding of rats in the TN group resulted in a significant 35.5% increase in IL-17 expression compared with rats in the normal control group. This result is similar to that of Che et al. [52], who showed that adult male C57BL/6 mice fed a high-fat diet strongly expressed IL-17 levels. In the same study, administration of the 200 mg/Pc aqueous extract of *Cirsium japonicum* lowered IL-17 expression levels in mice. Duan et al. [53] demonstrated the efficacy of *Dahuang Zhechong* pills in

reducing IL-17-secreting Th17 cells in NAFLD patients. Rats on the atherogenic diet had elevated levels of IL-17 due to hepatocyte damage. Fat accumulation in the liver can lead to the release by macrophages, of certain cytokines such as IL-6 and TNF alpha [54]. When fat accumulates in the liver, IL-17 stimulates kupffer cells to secrete cytokines such as IL-6, which in turn, when associated with TGF-β, stimulates the differentiation and activation of Th17 cells, which in turn produces IL-17 to trigger the inflammatory process *via* neutrophil infiltration [55]. Treatment with EthP$_{250}$ and PP$_{250}$ significantly reduced IL-17 expression by 50.25% and 100% respectively, compared with the TN group. This could be due to a decrease in IL-17 receptors on Th17 cells, or to inhibition of IL-17 secretion by macrophages. Also, progression from simple steatosis to non-alcoholic hepatic steatosis is characterized by increased accumulation of Th17 cells [56, 57]. Results showed that serum IL-17 expression was significantly reduced in the PP$_{250}$ group by 100% compared to the EthP$_{250}$ group. Administration of Propolis to rats on the atherogenic diet lowered IL-17 expression levels and thus reduced or ameliorated inflammation.

The atherogenic diet significantly reduced nitrite levels by 2.38% in the TN group compared with the normal control group. In contrast, treatment with EthP$_{250}$ and PP$_{250}$ significantly increased aortic nitrite levels by 1.38% and 1.63% respectively, compared with the TN group.

Treatment with EthP$_{250}$ and PP$_{250}$ significantly reduced IL-17 expression by 50.25% and 100% respectively, compared with the TN group. This could be due to a decrease in IL-17 receptors on Th17 cells, or to inhibition of IL-17 secretion by macrophages. Also, progression from simple steatosis to non-alcoholic hepatic steatosis is characterized by increased accumulation of Th17 cells [56, 57]. Results showed that serum IL-17 expression was significantly reduced in the PP$_{250}$ group by 100% compared to the EthP$_{250}$ group. Administration of Propolis to rats on the atherogenic diet lowered IL-17 expression levels and thus reduced or ameliorated inflammation. Sokeng et al. [57] demonstrated that the effect of Arachic Acid Ethyl Ester Isolated from Propolis has Anti-Inflammatory and Analgesic.

4.7. Effect of Hydroethanolic Extract and Fraction Powder ≤ 125μm of Propolis on Some Biomarkers

MDA is the end product of the ROS peroxidation reaction on lipids. It is therefore used as an indicator of free radical damage to cells [59]. Excess fat in hepatocytes can increase the beta-oxidation capacity of mitochondria and consequently incomplete lipid oxidation and increased free radical production [60].

The TN group showed a significant increase in MDA of 49.97% and 55.13% respectively in the kidney and heart in the TN group compared with the normal control. This increase is

thought to be due to ROS-mediated peroxidation of cell membrane lipids [60].

Rats given EthP$_{250}$ and PP$_{250}$ showed a significant decrease in MDA levels in the liver by 55.12% and 76.09% respectively; in the kidney by 57.24% and 63.71% in the heart, these values were 65.40% and 66.48% respectively; in the aorta by 46.57% and 48.53%. Propolis administration improved MDA levels in treated rats, opposing lipid peroxidation and preventing free radical damage [61].

This result proves that Propolis, with its wealth of bioactive compounds such as polyphenols, reduced the production of free radicals in the liver by neutralizing them, thus preventing them from destroying membrane lipids. Our results are similar to those of Amirinejad et al. [62] who showed that the hydroalcoholic extract of spinach ameliorated oxidative stress in NADFL by reducing MDA levels.

However, administration of PP$_{250}$ significantly increased MDA levels by 72.43% compared with EthP$_{250}$, due to the high concentration of bioactive compounds present in Propolis powder.

Oxidative stress is the imbalance between pro-oxidants and antioxidants in favor of pro-oxidants [63]. Antioxidants refer to all substances that, in low concentrations relative to the oxidizable substrate, delay or inhibit oxidation of the substrate [64]. Feeding rats an atherogenic diet for 45 days increased organ levels of reduced glutathione (GSH) and SOD. This increase is thought to be because when the liver is attacked by lipid accumulation, free radicals are produced. In response to this aggression, the body is predisposed to a cascade of antioxidant secretions to neutralize the free radicals generated by the atherogenic diet. The role of reduced glutathione (GSH) is to trap superoxide radicals and protect protein thiol groups from oxidation [65].

5. Conclusion and Perspectives

This study aimed to evaluate the preventive effects and anti-inflammatory activity of Propolis on atherogenic diet-induced non-alcoholic fatty liver disease in Wistar rats.

The *in vitro* antioxidant activity demonstrated that Propolis was very rich in phenolic compounds, which increased with Propolis concentration. A higher concentration of Zinc, Iron, Calcium, Manganese, and Vitamin C in the hydroethanolic extract compared to the powder fraction ≤ 125 μm of Propolis. Administration of the hydroethanolic extract and the Propolis powder fraction ≤ 125 μm in rats on an atherogenic diet limited liver damage by lowering transaminase levels. Propolis reduced fat accumulation in the liver by lowering total cholesterol, TG, and LDL-c levels and increasing HDL-c levels in rat serum. Anti-inflammatory activity was reduced by decreased IL-17 expression levels after administration of the hydroethanolic extract and the powder fraction ≤ 125 μm of Propolis. However, the most striking effects were obtained

with the Propolis powder fraction ≤ 125 µm. Propolis was shown to protect rats against non-alcoholic hepatic steatosis and to have anti-inflammatory activity. These results suggest that Propolis could be a promising adjunct in the management of non-alcoholic fatty liver, confirming its use in traditional medicine. However, further studies are needed to better understand Propolis' mechanisms of action and assess its short- and long-term deleterious effects.

Abbreviations

CDSp	Pulverization and Controlled Differential Screening Process
PP	The Powder Fraction≤ 125µm
EthP	Propolis Hydroethanolic Extract
ND	Normal Diet
AD	Atherogenic Diet
AST	Aspartate Aminotransferase
ALT	Alanine Aminotransferase
TC	Total Cholesterol
TG	Triglycerides
LDL	Low-Density Lipoprotein
HDL	High-Density Lipoprotein
VLDL	Very Low-Density Lipoprotein
TAC	Total Antioxidant Capacity
DPPH	-2,2-Diphenyl-1-Picrylhydrazyl
FRAP	Ferric Reducing Antioxidant Power
ROS	Reactive Oxygen Species
NO	Nitric Oxide
GSH	Reduced Glutathione
MDA	Malondialdehyde
SOD	Superoxide Dismutase
NADFLD	Nonalcoholic Fatty Liver Disease
Il-17	Interleukin 17
Th17	T-helper 17
TNF	Tumor Necrosis Factor
TGF-β	Transforming Growth Factor β

Data Availability Statement

All data are available on request from the corresponding author.

Conflicts of Interest

The authors declare no conflict of interest.

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