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RESEARCH ARTICLE

# *Apium graveolens* Aqueous Extract Reduced Cardiovascular Diseases and Inflammatory Biomarkers Expression in High-Fat Diet-Fed BALB/C Mice

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## Abstract

**Background and Aims**: Cardiovascular disease prevention has always been a high goal. The goal of this study is to investigate if *Apium graveolens* has any influence on cardiovascular disease risk factors, biomarkers, and inflammatory biomarkers in male BALB/c mice that have been given a high-fat diet. **Methods**: *Apium graveolens* aqueous extract was given to male BALB/c mice, and they were either fed a standard pellet or a diet composed of cholesterol (0.15%), sodium cholate (0.5%), and pure coconut oil (21%) for 12 weeks. Serum fasting glucose, a lipid profile, liver function tests, and cardiac indicators were used to evaluate the extract's anti-dyslipidemic, hypoglycemic, hepatoprotective, and cardioprotective characteristics. Antioxidant enzyme markers in tissues were also evaluated. To evaluate inflammatory and CVD biomarkers in cardiac tissue, RT-qPCR and ELISA were used. An unpaired t-test assessed group differences. $P < 0.05$ showed significance. **Results**: The HFD control group exhibited considerably higher levels of blood glucose, lipid profile, hepatic indicators, inflammatory and cardiac markers, and lower levels of HDL-C and antioxidant enzymes. When administered orally, an aqueous extract of *Apium graveolens* significantly reduced blood glucose levels. Serum lipids and liver indicators returned to near-normal levels. In addition to a considerable reduction in MDA levels, treated mice showed a large increases in catalase and reduced glutathione activities. Inflammatory and cardiovascular disease biomarker expression was reduced in the extract-treated groups. **Conclusions**: *Apium graveolens* consumption may help reduce the risk of cardiovascular disorders.

**Keywords:** *Apium graveolens*, Biomarkers, Cardiovascular Diseases (CVD), Inflammatory Biomarker, Risk Factors, Serum, Tissue Analysis

## 1. Introduction

The mortality rate caused by Cardiovascular Disease (CVD) is a big concern all over the world[1]. Cardiovascular disease involves heart and circulation dysfunctions[2]. Circulatory disorders include angina, heart attack, atherosclerosis, hypertension, stroke, and peripheral vascular disease[3]. Saturated fat, cholesterol, stress, cigarette use, physical inactivity, metabolic syndrome, atherosclerosis and hypertension all contribute to cardiovascular disease[4-6]. Metabolic syndrome is caused by abnormal cholesterol levels, elevated glucose levels, glucose intolerance, elevated blood pressure, fat accumulation, and obesity[7]. Several risk factors raise the risk of cardiovascular disease or stroke[7]. Oxidative stress from metabolic disorders causes CVD (i.e., increased generation of free radicals or a shortage of enzymatic or non-enzymatic antioxidants). Most CVD risk factors

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increase ROS[8]. ROS oxidize low-density lipoproteins, forming arterial plaques[9]. Humans have several antioxidant enzymes[10]; a reduction in an endogenous antioxidant enzyme has been linked to heart disease[11]. Reducing oxidative stress improves CVD treatment since ROS is critical to heart pathophysiology.

Atherosclerosis causes most CVD[12]. Atherosclerosis is coronary artery plaque accumulation. Endothelial dysfunction, vascular inflammation and fat, cholesterol, calcium, and cellular debris in big and medium-sized artery intima characterize this multifactorial inflammatory disease[13]. In early atherosclerosis, pro-inflammatory cytokines raise the risk of cardiovascular disease[14,15]. HFD patients had elevated CRP levels and several other inflammatory markers[16]. The development and progression of atherosclerosis, as well as higher C-reactive protein levels and CVD, have all been linked to metabolic syndrome and upregulation of inflammatory markers such as TNF-α, and others[17,18]. TNF-α decreases NO bioavailability and NOS levels, thereby reducing endothelium-mediated vasorelaxation[19,20]. IL-1 increases the rates at which matrix metalloprotease enzymes cause plaque to rupture, which can contribute to cardiovascular events[21]. These cytokines cause inflammatory cells to release IL-6, which raises hepatic CRP generation and accelerates the arterial wall inflammatory cascade[22]. Inflammation increases the risk of cardiovascular illness, so treating it may reduce the chance of sudden cardiac arrest.

Because atherosclerotic CVD develops in childhood, screening and early detection are possible. Several CVD biomarkers and inflammatory biomarkers have been reported so far[23]. We evaluated three biomarkers for cardiovascular disease: Creatine Kinases (CK), Cardiac Troponin I (CTN-I), and Natriuretic Peptides (NPs). These indicators help diagnose and treat different cardiac conditions[24]. CK fuels the brain, heart, and skeletal muscles. Hypertension increases due to increased ADP consumption and CK inhibition, which lowers blood pressure[25,26]. High serum CK levels are also correlated with dyslipidemia (hypercholesterolemia, hypertriglyceridemia, and low-HDL cholesterolemia). Hence, increased blood CK levels may better predict MI in dyslipidemia[27]. The actin and myosin binding activity of the skeletal and cardiac muscle is inhibited by troponins T, I and C. Troponin C is expressed in both skeletal and cardiac muscle, making it a less useful cardiac-specific marker than troponin T and I. However, CTN-I may aid in infarction detection because to its high sensitivity and specificity for myocardial injury[28-30]. Cardiomyocytes release cardiac troponin into the bloodstream proportionally to heart injury (by ischemia or a variety of other reasons)[31,32]. Mammals have three NP genes: *NPPA* (ANP), *NPPB* (BNP), and *NPPC* (CNP)[33,34]. The atria express the most NPPA and NPPB genes[35]. This study focused on *NPPA* and ANP because of their stronger physiological influence on cardio-renal function. ANP activates cGMP-dependent Protein Kinases (PKGs) to enhance natriuresis and diuresis, block juxtaglomerular cell renin secretion, and decrease proximal tubule and collecting duct salt and water reabsorption[36,37]. Hence, NPs play a crucial role in controlling blood pressure and can act as biomarkers for hypertension, a risk factor for CVDs. ST2 (Suppression of Tumorigenicity 2), a recently discovered biomarker can accurately predict the outcome for patients with CVDs[38]. Cardiac myocytes and fibroblasts express two types of ST2, one transmembrane or cellular (ST2L) and one soluble and blood-circulating (sST2)[39]. Like IL-1, damaged cells release IL-33, a cytokine with an ST2 receptor. Recent research shows that IL-33 protects against atherosclerosis, Type 2 diabetes, obesity, and cardiac remodeling[40]. IL-33's IL-5 and ox-LDL antibody stimulation reduces atherosclerosis. IL-33 reduced body fat, fasting glucose, insulin, and glucose tolerance in animals[41]. In animal studies, IL-33 and ST2L reduce myocardial fibrosis, hypertrophy, and apoptosis and increase function[42]. IL-33 with sST2 loses its cardioprotective function[43]. During oxidative stress or injury, cardiac fibroblasts, cardiomyocytes, and endothelial cells may generate sST2[44,45]. Hence, myocardial infarction and heart failure biomarkers include bloodstream sST2[42]. Serum Paraoxonase 1 also indicates cardiovascular disease (PON1). The three Paraoxonases in human serum - PON1, PON2 and PON3 - are most investigated[46]. It is predominantly generated in the liver and circulates in serum with HDL (HDL)[47]. PON1 inhibits LDL oxidation in vitro[48]. PON1 defends against free radicals, inflammation, cell death, clot formation, platelet adhesion, and lipid modification[49]. PON1 deficiency increases cardiovascular disease risk[48]. While PON1 synthesis protects against atherosclerosis in animals, PON1 deficiency increases the risk of atherosclerosis[50]. Hence, CVD treatment requires endothelium-protecting, anti-inflammatory, and antioxidant substances.

Avoiding risk factors prevents CVD. Dietary and herbal treatments for cardiovascular disease are becoming more popular. Several herbal supplements lower LDL cholesterol and increase HDL "good cholesterol" while protecting against lipid oxidation[51]. Traditional, herbal, and alternative medicine has identified about two thousand cardiovascular disease-treating plants[52]. *Apium graveolens* is a herb. It belongs to the Apiaceae family, *Apium* genus, *Apium* species (*gravolens*), and Magnoliophyta division (Plantae). Celery - *Apium graveolens* - has various names in many languages (E.g., in Persian: *Karaf*; Spanish: *Apio*; German: *Sellerie*; Arabic: *Alkarafs*, etc). Celery plants grow to 100 centimeters. It's strong-smelling and durable. Triangular diamond or spear-shaped leaves with saw teeth or lobe-shaped margins are 5-50 mm long. Although it's European, it's everywhere. Celery has well-known antioxidant, anti-inflammatory, and antifungal properties[53]. In mice, *Apium graveolens* protects various tissues and organs from free radical damage. Nonetheless, celery's cardioprotective potential and effects on cardiovascular risk factors and inflammatory biomarkers are the focus of the current research.

## 2. Materials and Methodology

### 2.1 Chemicals

TCA, TBA, MDA, and HCl were acquired from Sisco Research Laboratories (SRL), Bombay, India, as were hydrogen peroxide, Bradford reagent, potassium dichromate, DTNB (Ellman's reagent), and hydroxylamine. The Coral clinical system was used to acquire assay kits for measuring blood glucose, cholesterol, triglycerides, creatine kinase, creatine phosphokinase-MB, alanine aminotransferase, gamma glutamic acid, uric acid, bilirubin, lactate dehydrogenase, and high-density lipoprotein cholesterol. Wuhan Fine Biotech Co., Ltd., was where we got our ELISA kits for ANP and CTN-I.

### 2.2 Plant Material

Celery leaves (*Apium graveolens*) were obtained fresh from the market in Iewduh, Shillong, Meghalaya, India. Following washing in distilled water and drying at 25°C, the leaves were chopped and dried in a 40°C oven for three days. The dried leaves were then powdered using an electric blender and stored at 4°C in an airtight vial.

### 2.3 Preparation of Extract

The plant powder was extracted with distilled water at 1:10 (powder/solvent) with occasional shaking for 24 hrs. First, the extract was filtered using a muslin cloth, and then, after that, it was filtered through Whatman No. 1 filter paper. The filtrate was lyophilized using a rotary evaporator. Using sterile water, the leftovers were refrigerated at 4°C.

### 2.4 Experimental Animals

The Pasteur Institute in Shillong, Meghalaya, India, provided adult Swiss albino mice weighing 20-30 g. Five mice were housed in each polyacrylic cage under controlled laboratory settings. They were given distilled water and a regular dry pellet meal (both sourced from Hindustan Lever in Kolkata, India). Mice were introduced to the controlled environment of the laboratory seven days before the experiment began. The procedures involving animals in the experiments met the standards set by the Institutional Animal Ethics Committee (IAEC).

### 2.5 Dosage Selection

In the first study, which was titled "**Determination of the safe dose of aqueous extract of *Apium graveolens* L. by acute and sub-acute toxicity study**," three doses of aqueous extract of *Apium graveolens* were selected for this study based on serum levels of liver enzyme markers. These doses were 50 mg/kg b.w, 100 mg/kg b.w and 200 mg/kg b.w.

## 3. Cardiovascular Diseases Induction

A total of 40 male BALB/c mice, all of which were Swiss albino and used in the study, were allocated at random into eight groups of four mice each, as follows:

Group 1- Normal pellet.

Group 2 (50 mg/kg b.w)- Normal pellet + *Apium graveolens* aqueous extract.

Group 3 (100 mg/kg b.w)- Normal pellet + *Apium graveolens* aqueous extract.

Group 4 (200 mg/kg b.w)- Normal pellet + *Apium graveolens* aqueous extract.

Group 5- High-fat diet (0.15% cholesterol, 0.5% sodium cholate and 21% fat: 91% saturated fat, 7% monounsaturated fat, and 2% polyunsaturated fat)[54].

Group 6 (50 mg/kg b.w)- High-fat diet + *Apium graveolens* aqueous extract.

Group 7 (100 mg/kg b.w)- High-fat diet + *Apium graveolens* aqueous extract.

Group 8 (200 mg/kg b.w)- High-fat diet + *Apium graveolens* aqueous extract.

The extract was given to each group orally by gavage for a total of 12 weeks.

## 4. Collection of Samples

On the last day of treatment, after the animals had fasted for 18 hours, they were put to death, and their blood was drawn via a retro-orbital sinus puncture while they were administered a small dose of anesthesia. Biochemical markers such as AST, ALT, LDH, uric acid, and Fasting Lipid Profile (FLP) were determined by separating serum at 3000 rpm for 10 minutes at 4°C in the control, HFD, and extract-treated groups. After separating the liver, brain, kidney, heart, and spleen, each organ was weighed using an analytical weight balance. After determining and comparing the relative organ weights of all of the groups, all of the significant organs were promptly frozen at -80°C so that they could be examined at a later time. The Relative Organ Weight (ROW) was calculated as ROW equals the absolute weight of the organs (gram) divided by the total body weight of the mice on the day of sacrifice multiplied by 100[55].

## 5. Biochemical Analysis

### 5.1 Serum Analysis

Using enzyme kits as directed by the manufacturer, lipid profile test and liver function test were measured in serum (Coral clinical system, India). The Friedewald[56] method was used to estimate the amount of VLDL-C and LDL-C. VLDL-C was found by dividing TAG by 5 and LDL-C was found by subtracting TC from (HDL+VLDLc). The following formulas were used to find the Atherogenic Index (AI), the Cardiac Risk Ratio (CRR), and the Atherogenic Coefficient (AC)[57].

AI = LogTG/HDL-C

CRR-I = TC/HDL-C

AC = TC-HDLc/HDLc

### 5.2 Tissue Oxidative Stress Markers

We made a homogenate of the liver, heart, brain, and kidney (1g) by using a Teflon homogenizer and mixing it in 0.1M Tris HCl buffer that had a pH of 7.3. The supernatant obtained after centrifugation at 4°C (3000 rpm) for 20 mins was examined to assess CAT[58], GSH[59], lipid peroxidation product (MDA)[60], and total protein[61]. This was done following the procedure.

### 5.3 Cardiac Gene Expression Analysis

TRIzol was employed to isolate total RNA (Life Technologies, USA). For each sample's total RNA analysis, we utilized the Qubit RNA Assay Kit. Following the manufacturer's instructions, cDNA was generated with an oligodT18 primer and M-MLV reverse transcriptase (Invitrogen, USA). qPCR was utilized to compare the levels of mRNA expression in the various treatment groups. With a 7500 Fast qPCR machine, the PowerUp SYBR Green Master Mix was utilized (Applied Biosystems). In the procedure, 1 µl of cDNA, 0.25 µl of reverse and forward primers, 6.25 µL of PowerUp SYBR Green Master mix, and the total volume was adjusted to 12.5 µL with nuclease-free water solution was used. The mRNA levels of the target gene were normalized to those of the housekeeping gene β-ACTIN in the same sample. Compared to the baseline of control mice, fold changes are presented. As shown in Table 1, we created primers using a verified online tool, Primer 3[62,63].

### 5.4 Cardiac Protein Analysis

Pre-cooled 0.01M PBS buffer (pH 7.4) was used to homogenize the heart and kidney. The supernatant was obtained by centrifuging the homogenates for 5 minutes at 5000×g after homogenization. To measure the total protein concentration, the Bradford assay was performed. The ELISA kit manufacturer's instructions were followed to determine the amounts of cardiac-specific proteins like ANP and CTN-I in control, HFD and treated mice (Wuhan Fine Biotech Co., Ltd).

## 6. Statistical Analysis

GraphPad Prism 8.0 was used for statistical analysis. The mean and standard deviation summarized the data (SEM). Unpaired t-tests assessed group differences. ($p < 0.05$) was significant.

**Table 1.** Characteristics of primers

| Genes | Primers | | Size (bp) |
|---|---|---|---|
| Name | Forward sequences (5' to 3') | Reverse sequences (3' to 5') | No. of bases |
| *CRP* | TCTGCACAAGGGCTACACTG | AAACATTGGGGCTGAGTGTC | 20 |
| *TNF-α* | TCTCATCAGTTCTATGGCCC | GGGAGTAGACAAGGTACAAC | 20 |
| *IL-6* | GTTCTCTGGGAAATCGTGGA | TGTACTCCAGGTAGCTATGG | 20 |
| *IL-1β* | TTGACGGACCCCAAAAGATG | AGAAGGTGCTCATGTCCTCA | 20 |
| *ST-2* | CCAGCCAGAGTGGAAGACTC | CAGGTCAATTGTTGGACACG | 20 |
| *IL-33* | ATCACGGCAGAATCATCGAG | CTTATGGTGAGGCCAGAACG | 20 |
| *PON-1* | CAGCCTGTCCATCTGTCTCA | CACCCGTCTCGATTCCTTTA | 20 |
| *CK-M* | GCTTCGCGATAAGGAGACAC | GGATGATGGGATCAAACAGG | 20 |
| *CK-B* | CCCTTCTCCAACAGCCATAA | CGGATTGTCTACGCCAGTCT | 20 |
| *CTN-I* | TAAGATCTCCGCCTCCAGAA | CGGCATAAGTCCTGAAGCTC | 20 |
| *ANP* | ATCTGCCCTCTTGAAAAGCA | ACACACCACAAGGGCTTAGG | 20 |
| *β-ACTIN* | TGTTACCAACTGGGACGACA | GGGGTGTTGAAGGTCTCAAA | 20 |

*CRP*= C-Reactive protein, *TNF-α*= Tumor necrosis factor, *IL-6/1β*= Interleukine, *ST-2*= Soluble transmembrane, *Il-33*= Interleukine, *PON-1*= Paraoxonase, CK= Creatine kinase, CTN-I= Cardiac Troponin, *ANP*= Atrial Natriuretic peptide.

# 7. Results

## 7.1 Effect of Oral Administration of an Aqueous Extract of *Apium graveolens* on Body Weight in Control, HFD and Treated Mice

The measures of weight that were obtained before, throughout, and after the treatment period are given in Table 2 and Figure 1, respectively, while the initial weight and final weight are shown in Figure 2.

When weights were taken on day 1, they were compared to the weights taken at the end of each animal's treatment period (12 weeks). The control ($p = 0.0004$), 50 mg/kg ($p = 0.001$), and 100 mg/kg ($p = 0.027$) groups all gained significant amounts of body weight (4.8 ± 0.1 gram), (2.1 ± 0.1 gram), and (1.4 ± 0.4 gram) throughout treatment (Figure 2), whereas the extract at a dose of 200 mg/kg resulted in an insignificant ($p = 0.6086$) weight loss (-0.5 ± 0.9 gram) depicted in Table 1. Similarly, it was observed that in HFD ($p = 0.0004$) treated group, the body weight significantly increased (5.4 ± 0.1gram) in mice during the experimentation (Figure 2). However, 12 weeks of oral treatment of *Apium graveolens* aqueous extract at doses of 50 mg/kg (4.3 ± 0.1 gram) and 100 mg/

**Table 2.** Effect of HFD and oral administration of an aqueous extract of *Apium graveolens* on body weight (g) in *Swiss* albino mice for 12 weeks

| Treatment | Weekly weight of BALB/c mice (g) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| mg/kg b.w. | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 | Week 6 | Week 7 | Week 8 | Week 9 | Week 10 | Week 11 | Week 12 | Weight gain or loss |
| Control | 20.9 ± 0.61 | 21.1 ± 0.58 | 21.5 ± 0.5 | 21.9 ± 0.4 | 22.6 ± 0.6 | 23.2 ± 0.4 | 24.03 ± 0.5 | 24.6 ± 0.7 | 24.8 ± 0.8 | 25.1 ± 0.8 | 25.4 ± 0.7 | 25.8 ± 0.7 | ↑4.8 ± 0.1 |
| 50 | 21.7 ± 0.2 | 21.8 ± 0.3 | 22.7 ± 0.4 | 23.2 ± 0.4 | 22.9 ± 0.4 | 23.1 ± 0.5 | 23.1 ± 0.3 | 23.1 ± 0.3 | 23.3 ± 0.4 | 23.5 ± 0.3 | 23.8 ± 0.3 | 23.9 ± 0.4 | ↑2.1± 0.1 |
| 100 | 21.2 ± 0.7 | 21.7 ± 0.4 | 21.9 ± 0.5 | 22.03 ± 0.4 | 22.1 ± 0.3 | 22.2 ± 0.4 | 22.2 ± 0.3 | 22.06 ± 0.3 | 22.1 ± 0.5 | 21.8 ± 0.2 | 21.9 ± 0.2 | 22.6 ± 0.2 | ↑1.4±0.4 |
| 200 | 23.06 ± 0.6 | 23.03 ± 0.7 | 23.0 ± 0.6 | 23.1 ± 0.6 | 23.1 ± 0.5 | 23.1 ± 0.5 | 22.8 ± 0.6 | 22.5 ± 0.5 | 22.6 ± 0.6 | 21.9 ± 0.5 | 22.6 ± 0.3 | 22. 5 ± 0.8 | ↓0.5± 0.9 |
| HFD | 22.4 ± 0.7 | 22.4 ± 0.8 | 22.9 ± 0.8 | 23.7 ± 0.4 | 24.1 ± 0.5 | 24.8 ± 0.5 | 25.2 ± 0.5 | 26.2 ± 0.4 | 26.6 ± 0.61 | 27.03 ± 0.6 | 27.5 ± 0.6 | 27.8 ± 0.6 | ↑5.4 ±0.1 |
| HFD +50 | 23.8 ± 0.2 | 23.9 ± 0.2 | 24.06 ± 0.2 | 24.3 ± 0.3 | 24.2 ± 0.6 | 24.2 ± 0.6 | 24.8 ± 0.4 | 25.6 ± 0.4 | 26.1 ± 0.4 | 26.8 ± 0.2 | 27.3 ± 0.1 | 28.1 ± 0.3 | ↑4.3± 0.1 |
| HFD +100 | 23.5 ± 0.6 | 24.1 ± 1.1 | 24.3 ± 1.05 | 24.2 ± 1.12 | 23.8 ± 1.1 | 23.9 ± 1.04 | 24.7 ± 1.6 | 24.3 ± 1.4 | 24.2 ± 1.3 | 23.8 ± 1.2 | 23.5 ± 1.2 | 25.3 ± 0.6 | ↑1.8 ±0.01 |
| HFD +200 | 22.8 ± 1.2 | 23.2 ± 0.8 | 23.6 ± 0.7 | 23.8 ± 0.7 | 23.8 ± 0.6 | 24.06 ± 0.4 | 23.8 ± 0.7 | 23.2 ± 0.7 | 23.0 ± 0.7 | 22.8 ± 0.6 | 22.6 ± 0.7 | 23.4 ± 0.5 | ↑0.6 ±0.1 |

The data are expressed as mean ± SEM (n = number of animals in each group = 4).↑↓ indicate gain or loss in body weight

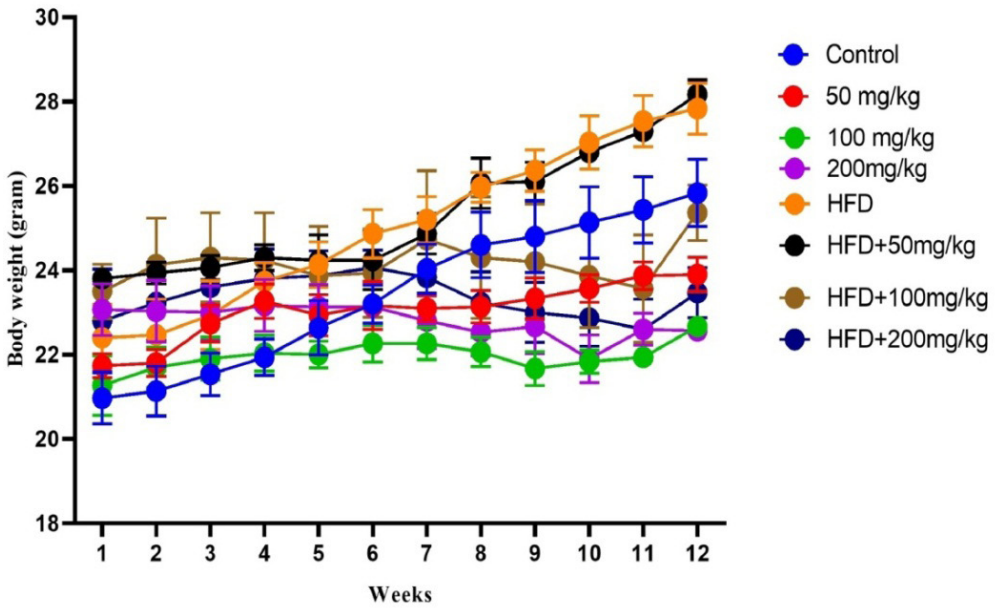



**Figure 1.** Effect of HFD and oral administration of an aqueous extract of *Apium graveolens* on body weight (g) in *Swiss* albino mice for 12 weeks.

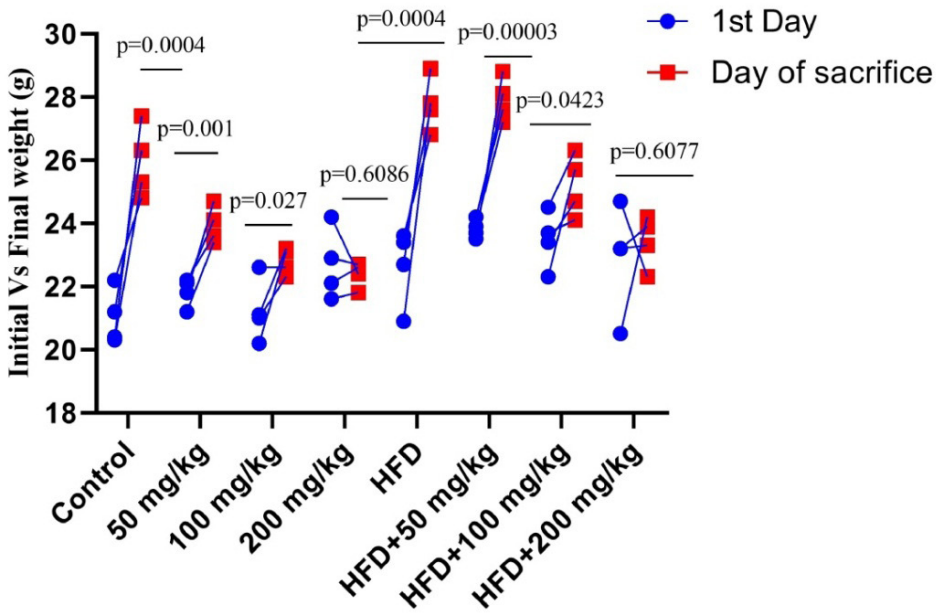



**Figure 2.** Effect of HFD and oral administration of an aqueous extract of *Apium graveolens* on initial and final body weight (g) in *Swiss* albino mice for 12 weeks. (Analyzed by multiple t-tests).

kg (1.8 ± 0.01 gram) resulted in a progressive decrease in body weight. However, the HFD+200 mg/kg (p = 0.6077) group did not display statistically significant changes in body weight (0.6 ± 0.1 gram) at the end of the treatment period (Figure 2). Thus, both the control and HFD groups gained weight during the treatment period, and all interventions resulted in a consistent weekly weight decrease following the treatment period.

## 7.2 Effect of Oral Administration of an Aqueous Extract of *Apium graveolens* on Absolute Organ Weight and Relative Organ Weight in Control, HFD and Treated Mice

The absolute organ weight of each organ was determined after the end of the treatment period is shown in Table 3 and Figure 3.

Mice given 200 mg/kg experienced a significant decrease in liver weight ($p = 0.0207$), while mice given an HFD experienced a significant increase in liver weight ($p = 0.0005$). However, compared to both the control and HFD groups, there is a significant reduction in liver weight in the HFD + 200 mg/kg group ($p < 0.0001$) ($p < 0.0191$) as shown in Figure 3. When comparing the 200 mg/kg ($p = 0.1733$), HFD ($p = 0.0826$), HFD + 200 mg/kg ($p = 0.7259$), and HFD + 200 mg/kg ($p = 0.2232$), HFD + 200 mg/kg ($p = 0.6190$) groups to the control group, there were no statistically significant differences in the weights of the kidney, heart, brain, or spleen (Figure 3). Similarly, the kidney ($p = 0.1299$), heart ($p = 0.1489$), brain ($p = 0.2005$), and spleen ($p = 0.6190$) of the HFD + 200 mg/kg group are not significantly different from those of the HFD group.

The relative change in organ weight (Absolute organ weight (g)/Mice body weight on sacrifice day 100) is depicted in Figure 4 and Table 3. Liver weight was significantly higher in the 200 mg/kg ($p = 0.0115$) and HFD ($p = 0.0016$) groups compared to the control group, but in the HFD + 200 mg/kg ($p = 0.0191$) group, the opposite was the case. However, there are no statistically significant differences ($p = 0.3393$) in liver weight between the HFD + 200 mg/kg group and the control group. When comparing the 200 mg/kg ($p = 0.0702$), HFD ($p = 0.8324$), HFD + 200 mg/kg ($p = 0.3476$), HFD + 200 mg/kg ($p = 0.9837$), HFD + 200 mg/kg ($p = 0.8861$), and HFD + 200 mg/kg ($p = 0.1372$) groups to the control group, no statistically significant differences were seen in the kidney, heart, brain, or spleen (Figure 4). The kidneys ($p = 0.4356$), hearts ($p = 0.9064$), brains ($p = 0.2330$), and spleens ($p = 0.3320$) of those on the HFD + 200mg/kg diet show no statistically significant differences from those on the HFD control diet.

## 7.3 The Antihyperlipidemic and Anti-Atherogenic Activity of *Apium graveolens* in Control, HFD and Treated Mice

The results of the serum fasting lipid profile are summarized in Table 4 and Figure 5.

In HFD-fed mice, blood cholesterol ($p = 0.0003$), LDL-C ($p = 0.0003$), VLDL-C ($p = 0.0139$), triglyceride ($p = 0.0194$), AI ($p = 0.0020$), CRR ($p = 0.0001$) and AC ($p < 0.0001$) levels increased considerably, whereas HDL-C ($p = 0.0523$) declined dramatically as compared to the control group. Cholesterol ($p < 0.0001$), LDL-C ($p < 0.0001$), VLDL-C ($p = 0.0012$), triglyceride ($p = 0.0022$), AI ($p = 0.0123$), CRR ($p < 0.0001$), and AC ($p < 0.0001$) were all reduced by oral therapy with the aqueous extract at a dose of 200 mg/kg, whereas HDL-C ($p < 0.0001$) was increased (Figure 5). Similarly, the HFD + 200 mg/kg treated group showed significantly lower total cholesterol ($p = 0.0115$),

**Table 3.** Effect of HFD and oral administration of an aqueous extract of *Apium graveolens* on organ weight and relative organ weight (grams) in *Swiss* albino mice for 12 weeks

| ORGANS | ABSOLUTE ORGAN WEIGHT (g) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| TREATMENT | CONTROL | 50 mg/kg bw | 100 mg/kg bw | 200 mg/kg bw | HFD | HFD + 50 mg/kg bw | HFD + 100 mg/kg bw | HFD + 200 mg/kg bw |
| Liver | 1.4 ± 0.01 | 1.41 ± 0.03 | 1.36 ± 0.08 | 1.34 ± 0.05 | 1.78 ± 0.02 | 1.49 ± 0.22 | 1.33 ± 0.08 | 1.33 ± 0.11 |
| Kidney | 0.25 ± 0.04 | 0.25 ± 0.04 | 0.24 ± 0.08 | 0.23 ± 0.07 | 0.27 ± 0.08 | 0.26 ± 0.07 | 0.26 ± 0.08 | 0.24 ± 0.01 |
| Heart | 0.33 ± 0.03 | 0.32 ± 0.24 | 0.32 ± 0.12 | 0.32 ± 0.17 | 0.35 ± 0.09 | 0.3 ± 0.03 | 0.3 ± 0.01 | 0.3 ± 0.27 |
| Brain | 0.35 ± 0.02 | 0.37 ± 0.02 | 0.37 ± 0.02 | 0.36 ± 0.02 | 0.34 ± 0.01 | 0.33 ± 0.02 | 0.35 ± 0.01 | 0.32 ± 0.01 |
| Spleen | 0.12 ± 0.06 | 0.12 ± 0.05 | 0.12 ± 0.07 | 0.12 ± 0.08 | 0.14 ± 0.01 | 0.13 ± 0.08 | 0.139 ± 0.04 | 0.13 ± 0.07 |
| ORGANS | RELATIVE ORGAN WEIGHT (g) | | | | | | | |
| Liver | 5.45 ± 0.01 | 5.9 ± 0.05 | 6 ± 0.14 | 5.95 ± 0.01 | 6.4 ± 0.05 | 5.32 ± 0.15 | 5.27 ± 0.17 | 5.68 ± 0.18 |
| Kidney | 0.97 ± 0.01 | 1.07 ± 0.01 | 1.09 ± 0.03 | 1.05 ± 0.02 | 0.98 ± 0.03 | 0.94 ± 0.02 | 1.02 ± 0.05 | 1.05 ± 0.07 |
| Heart | 1.3 ± 0.05 | 1.35 ± 0.12 | 1.44 ± 0.04 | 1.44 ± 0.76 | 1.28 ± 0.04 | 1.09 ± 0.12 | 1.2 ± 0.04 | 1.3 ± 0.12 |
| Brain | 1.38 ± 0.1 | 1.57 ± 0.08 | 1.63 ± 0.09 | 1.6 ± 0.08 | 1.24 ± 0.06 | 1.2 ± 0.11 | 1.41 ± 0.06 | 1.37 ± 0.05 |
| Spleen | 0.49 ± 0.02 | 0.53 ± 0.02 | 0.55 ± 0.03 | 0.53 ± 0.03 | 0.5 ± 0.04 | 0.48 ± 0.03 | 0.54 ± 0.02 | 0.57 ± 0.03 |

The data are expressed as mean ± SEM (n = number of animals in each group = 4).

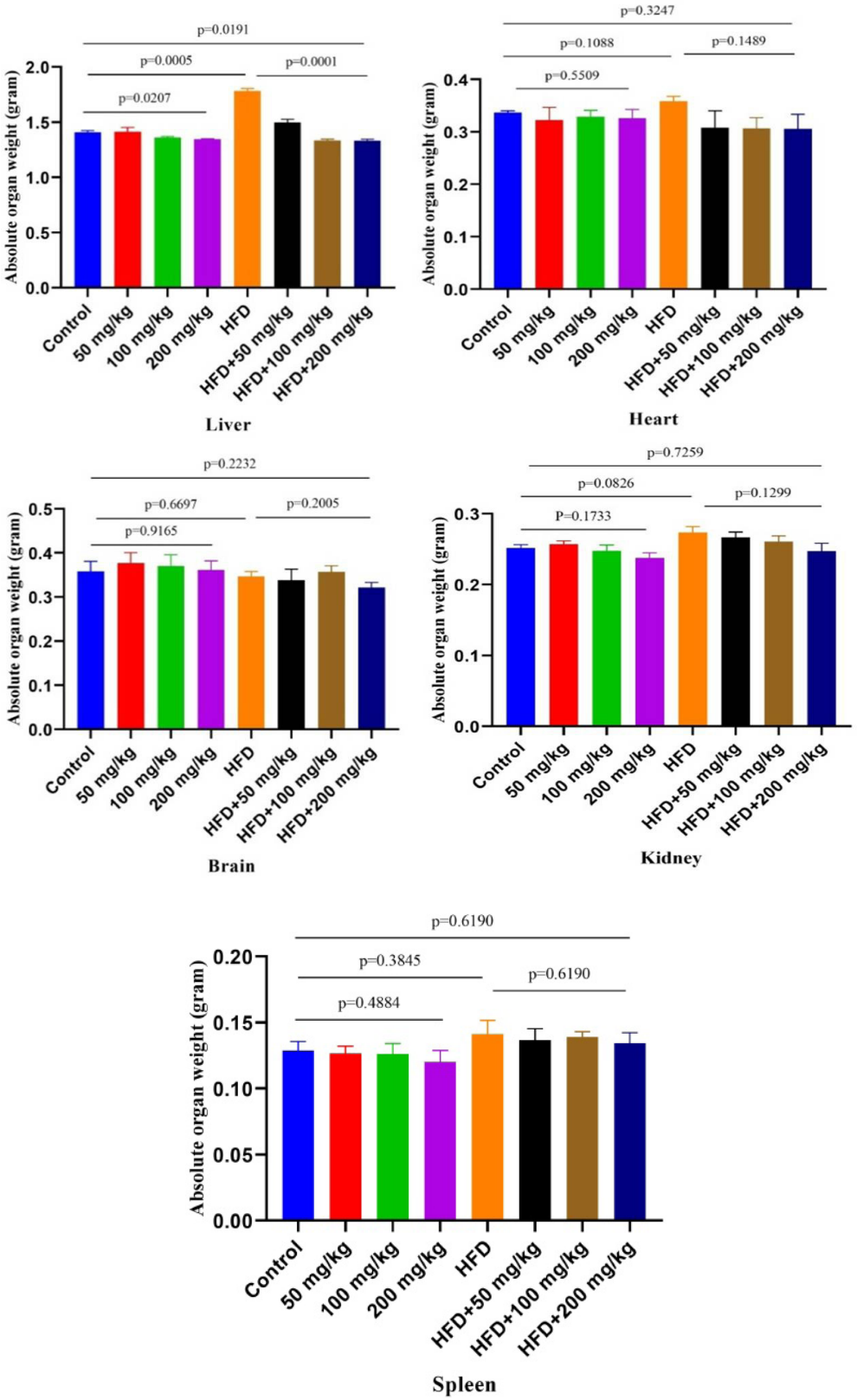



**Figure 3.** Effect of oral administration of *Apium graveolens* on absolute organ weight (grams) in control, HFD, and celery-treated *Swiss* albino mice for 12 weeks.

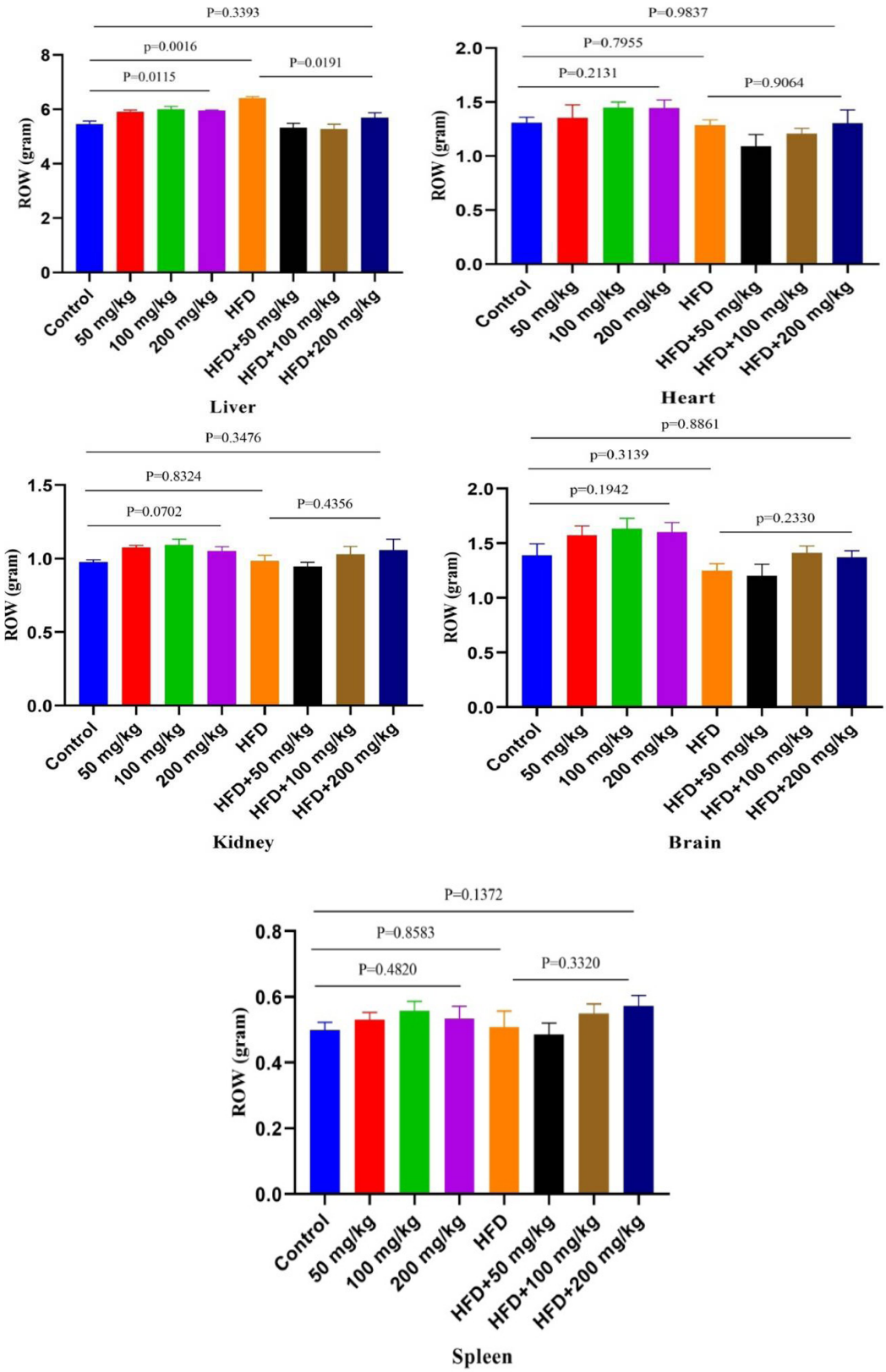



**Figure 4.** Effect of oral administration of *Apium graveolens* on relative organ weight (grams) in normal, HFD, and celery-treated *Swiss* albino mice for 12 weeks.

**Table 4.** The effect of HFD and oral supplementation of *Apium graveolens* on lipid profile, AI, CRR, and AC in *Swiss* albino mice for 12 weeks

| Parameters | Control | 50 mg/kg bw | 100 mg/kg bw | 200 mg/kg bw | HFD | HFD + 50 mg/kg bw | HFD + 100 mg/kg bw | HFD + 200 mg/kg bw |
|---|---|---|---|---|---|---|---|---|
| T. Cholesterol(mg/dL) | 127.2 ± 4.8 | 132.1 ± 2.0 | 120 ± 6.8 | 89.86 ± 2.4 | 172.3 ± 5.9 | 174.9 ± 9.7 | 160.1 ± 5.9 | 148.5 ± 4.1 |
| LDL-C (mg/dL) | 70.4 ± 4.5 | 67.9 ± 5.0 | 49.7 ± 9.6 | 15.76 ± 1.4 | 102.9 ± 4.0 | 52.2 ± 6.9 | 32.4 ± 7.1 | 11.57 ± 1.6 |
| TG-C (mg/dl) | 91.3 ± 5.8 | 101.5 ± 1.7 | 81.21 ± 6.1 | 40.6 ± 3.3 | 182.7 ± 1.3 | 193.04 ± 8.1 | 162.4 ± 6.2 | 121.8 ± 1.1 |
| VLDL-C (mg/dl) | 17.2 ± 1.9 | 10.8 ± 3.2 | 12.1 ± 2.3 | 6.469 ± 0.9 | 27.5 ± 3.9 | 31.05 ± 3.4 | 28.9 ± 2.7 | 20.9 ± 2.2 |
| HDL-C (mg/dl) | 44.2 ± 3.0 | 48.9 ± 1.7 | 60 ± 1.2 | 70.6 ± 2.7 | 34.7 ± 1.2 | 81.3 ± 6.2 | 103.1 ± 6.8 | 125.5 ± 5.3 |
| AI | 0.26 ± 0.1 | 0.10 ± 0.07 | 0.06 ± 0.1 | -0.37 ± 0.09 | 0.73 ± 0.08 | 0.27 ± 0.05 | 0.05 ± 0.1 | -0.07 ± 0.08 |
| CRR | 3.14 ± 0.2 | 2.64 ± 0.07 | 1.99 ± 0.1 | 1.34 ± 0.05 | 4.90 ± 0.08 | 2.17 ± 0.1 | 1.60 ± 0.15 | 1.11 ± 0.03 |
| AC | 2.144 ± 0.2( | 1.64 ± 0.07 | 0.99 ± 0.10 | 0.349 ± 0.05 | 3.939 ± 0.06 | 1.173 ± 0.19 | 0.608 ± 0.15 | 0.116 ± 0.03 |

The data are expressed as mean ± SEM (n = number of animals in each group = 4). TG= Triglyceride, LDL= Low Density Lipo protein, VLDL= Very Low Density Lipoprotein, HDL= High Density Lipoprotein, AI= Atherogenic index, CRR= Cardiac risk ratio, and AC= Atherogenic coefficient

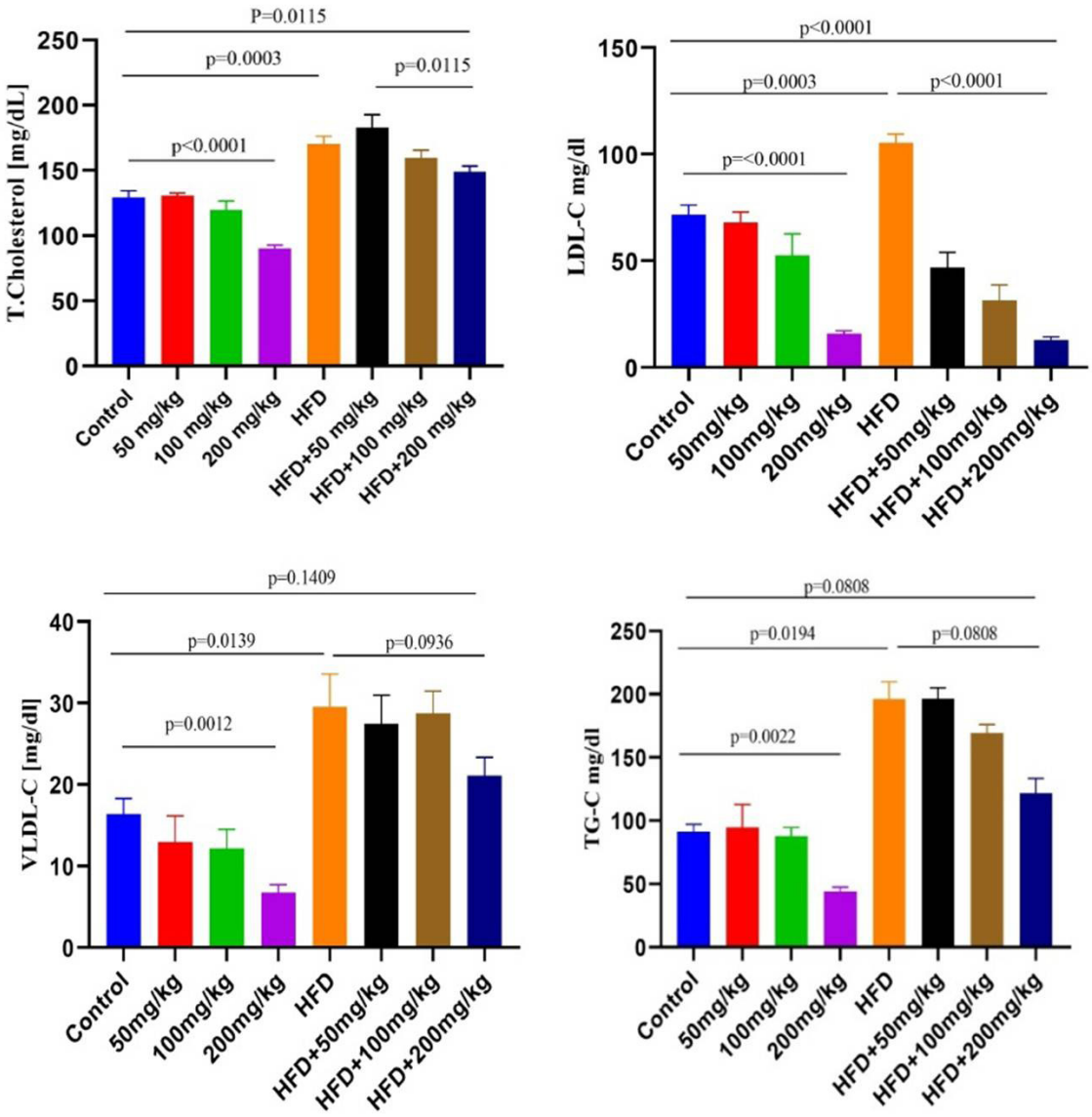



**Figure 5 to be continued...**

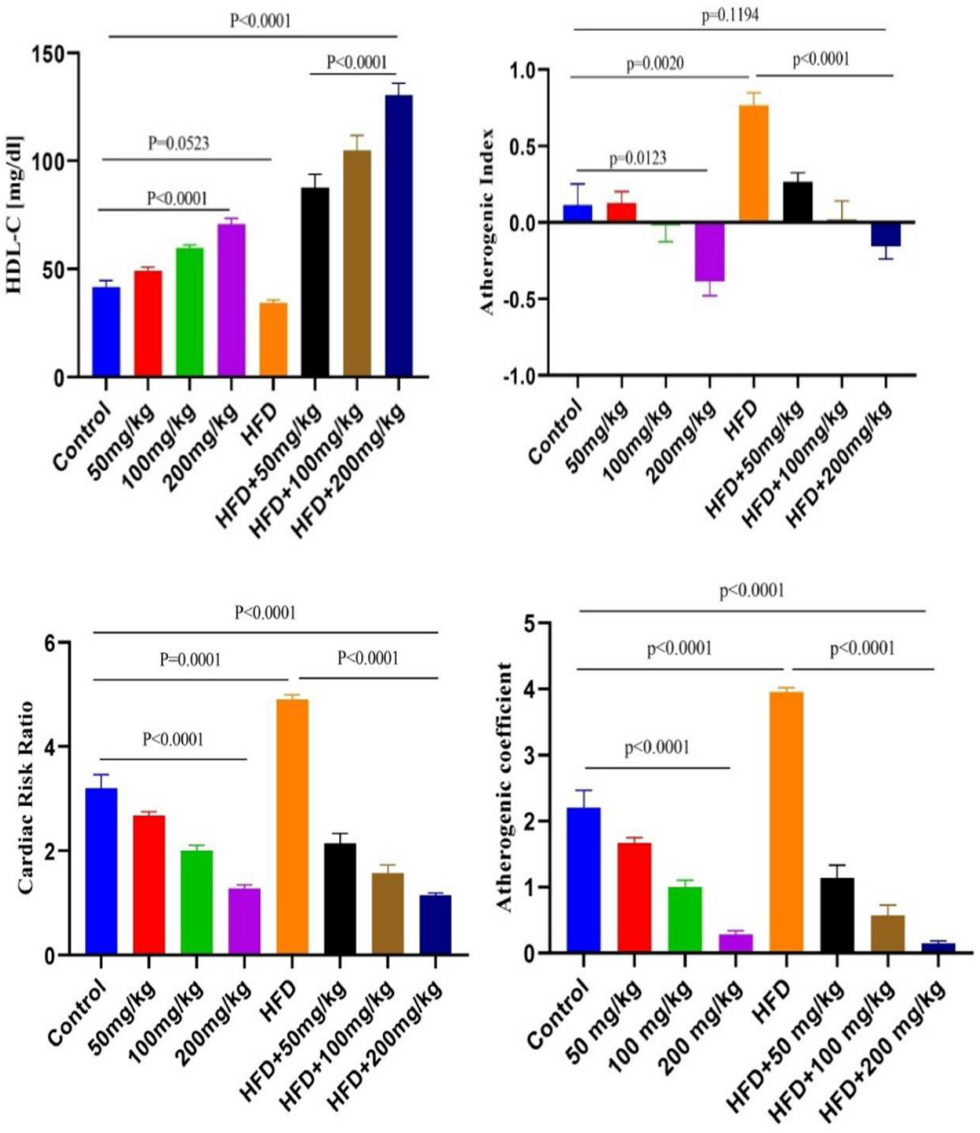



**Figure 5.** Effect of oral administration of *Apium graveolens* on lipid profile, AI, CRR, and AC in control, HFD, and celery-treated *Swiss* albino mice for 12 weeks. Total cholesterol, Triglycerides, Low-Density Lipoprotein, Very Low-Density Lipoprotein, High-Density Lipoprotein, AI = Atherogenic Index, CRR = Cardiac risk ratio and AC = Atherogenic coefficient.

LDL-C ($p < 0.0001$), AI ($p < 0.0001$), CRR ($p < 0.0001$), and AC ($p < 0.0001$) compared to the HFD control group, except VLDL-C ($p = 0.0936$) and triglyceride ($p = 0.0808$), and higher HDL-C ($p < 0.0001$) levels. In contrast, the HFD + 200 mg/kg treated group showed statistically significant increases in HDL-C ($p < 0.0001$) and decreases in cholesterol ($p = 0.0115$), LDL-C ($p < 0.0001$), CRR ($p < 0.0001$), and AC ($p < 0.0001$) when compared to the control group, except VLDL-C ($p = 0.1409$), triglyceride ($p = 0.0808$), and AI ($p = 0.1194$).

## 7.4 Effect of Oral Administration of an Aqueous Extract of *Apium graveolens* in Serum Hepatic Markers in Control, HFD and Treated Mice

The effects of *Apium graveolens* aqueous extract on several biochemical parameters in the control, HFD and celery-treated groups are summarized in Table 5 and Figure 6.

Only gamma-glutamyl transferase ($p = 0.5726$), total Bilirubin ($p = 0.0813$) and direct Bilirubin ($p = 0.1449$)

**Table 5.** Effect of HFD and oral administration of *Apium graveolens* on serum enzyme levels in *Swiss* albino mice for 12 weeks

| Parameters | Control | 50 mg/kg bw | 100 mg/kg bw | 200 mg/kg bw | HFD | HFD + 50 mg/kg bw | HFD + 100 mg/kg bw | HFD + 200 mg/kg bw |
|---|---|---|---|---|---|---|---|---|
| Glucose (mg/dL) | 16.1 ± 0.3 | 15.8 ± 0.1 | 14.5 ± 0.4 | 11.9 ± 1.0 | 19.5 ± 0.4 | 14.8 ± 0.1 | 10.3 ± 1.7 | 7.5 ± 0.5 |
| SGPT (U/mL) | 17.03 ± 0.12 | 16.6 ± 0.2 | 16.4 ± 0.3 | 15.5 ± 0.3 | 59.4 ± 4 | 28.3 ± 0.7 | 22.4 ± 0.6 | 20.1 ± 0.3 |
| SGOT (U/mL) | 45.2 ± 0.6 | 43.2 ± 0.5 | 34.7 ± 1.2 | 27.2 ± 2.7 | 114.2 ± 8.7 | 128.7 ± 2.0 | 99.7 ± 4.8 | 75.2 ± 6.9 |
| CK (U/L) | 11.6 ± 1.0 | 10.3 ± 0.4 | 8.3 ± 1.2 | 7.4 ± 1.0 | 16.6 ± 0.4 | 15.8 ± 0.4 | 11.6 ± 0.7 | 9.1 ± 0.4 |
| CK-MB (U/L) | 13.3 ± 0.3 | 11.6 ± 0.3 | 10.8 ± 0.8 | 8.3 ± 0.6 | 18.3 ± 0.5 | 17.4 ± 1.0 | 14.1 ± 0.9 | 11.6 ± 0.9 |
| γ-GT (U/L) | 3.1 ± 0.2 | 2.5 ± 0.3 | 2.3 ± 0.2 | 2.3 ± 0.4 | 6.1 ± 1.3 | 2.8 ± 0.1 | 3.08 ± 0.2 | 2.1 ± 0.3 |
| T.Bilirubin (mg/dL) | 0.13 ± 0.09 | 0.14 ± 0.007 | 0.14 ± 0.003 | 0.11 ± 0.007 | 0.16 ± 0.002 | 0.22 ± 0.01 | 0.16 ± 0.007 | 0.15 ± 0.003 |
| D.Bilirubin (mg/dL | 0.16 ± 0.08 | 0.16 ± 0.01 | 0.16 ± 0.01 | 0.15 ± 0.007 | 0.19 ± 0.005 | 0.18 ± 0.007 | 0.15 ± 0.007 | 0.12 ± 0.008 |
| Uric acid (mg/dL) | 0.27 ± 0.07 | 0.18 ± 0.03 | 0.12 ± 0.01 | 0.09 ± 0.01 | 0.43 ± 0.08 | 0.55 ± 0.03 | 0.27 ± 0.03 | 0.19 ± 0.04 |
| LDH (U/L) | 29.9 ± 0.9 | 30.9 ± 1.1 | 19.4 ± 2.2 | 19.9 ± 1.8 | 120.5 ± 6.3 | 70.5 ± 8.3 | 48.3 ± 8.8 | 34.9 ± 8.2 |

The data are expressed as mean ± SEM (n = number of animals in each group = 4). SGPT=Serum glutamic pyruvic transaminase, SGOT= Serum glutamic oxaloacetic, CK= Creatine kinase, γ-GT= Gamma glutamyl transferase, Total Bilirubin, Direct Bilirubin and LDH= Lactate dehydrogenase.

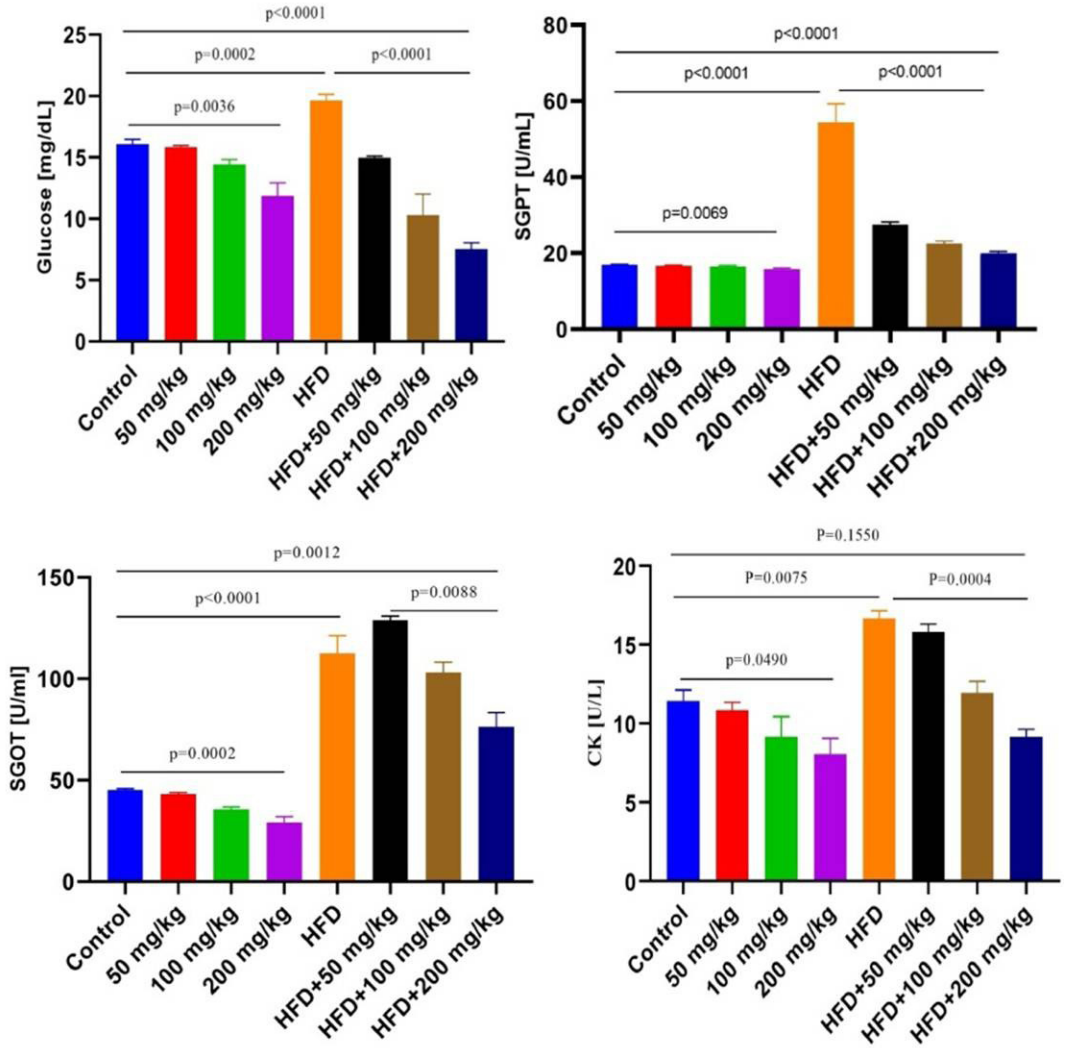



**Figure 6 to be continued...**

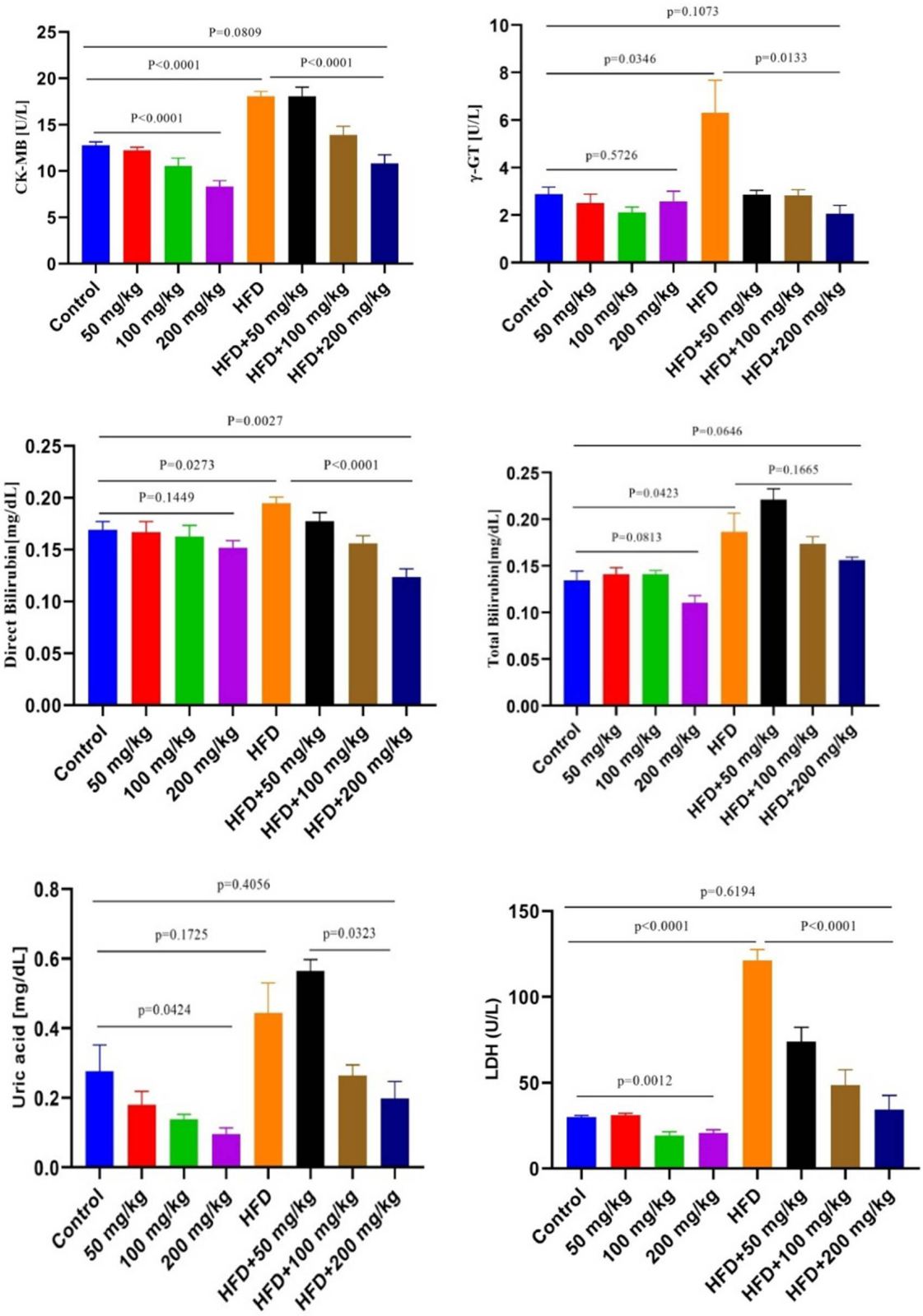



**Figure 6.** Effect of HFD and oral administration of *Apium graveolens* on serum enzyme levels in *Swiss* albino mice for 12 weeks. **(A).** Aspartate aminotransferase. **(B).** Alanine aminotransferase. **(C).** Lactate dehydrogenase. **(D).** CK = Creatine Kinase.

were found to be insignificantly decreased in the groups treated with 200 mg/kg of *Apium graveolens* aqueous extract, while significantly decreased in glucose ($p = 0.0036$), SGPT ($p < 0.0001$), SGOT ($p = 0.0002$), CK ($p = 0.0490$), CK-MB ($p < 0.0001$), uric acid ($p = 0.0424$)), and LDH ($p = 0.0012$) level when compared to the control group (Figure 6). When it came to the serum levels of glucose and liver enzymes, except for uric acid ($p = 0.1725$), all of the measured parameters increased considerably in mice fed HFD: glucose ($p = 0.0002$), SGPT ($p < 0.0001$), SGOT ($p = 0.0001$), CK ($p = 0.0075$), CK-MB ($p < 0.0001$), gamma-glutamyl transferase ($p = 0.0346$), total Bilirubin ($p = 0.0423$) and direct Bilirubin ($p = 0.0273$), and LDH ($p < 0.0001$) (Figure 6). Similarly, as compared to the HFD control group, the HFD + 200 mg/kg treated group revealed substantial reductions in glucose ($p < 0.0001$), SGPT ($p < 0.0001$), SGOT ($p = 0.0088$), CK ($p = 0.0004$), CK-B ($p < 0.0001$), CK-MB ($p < 0.0001$), gamma-glutamyl transferase ($p = 0.0133$), direct Bilirubin ($p < 0.0001$), uric acid ($p = 0.0323$), and LDH ($p < 0.0001$) except in total Bilirubin ($p = 0.1665$). Apart from a significant reduction in glucose ($p < 0.0001$), SGPT ($p < 0.0001$), SGOT ($p = 0.0012$), direct Bilirubin ($p = 0.0027$), the HFD + 200 mg/kg treated group also retained CK-B ($p = 0.0809$), CK-MB ($p = 0.0809$), gamma-glutamyl transferase ($p = 0.1073$), total Bilirubin ($p = 0.0646$), uric acid ($p = 0.4056$) and LDH ($p = 0.6194$) to about normal level when compared to the control group (Figure 6).

## 7.5 Effect of Oral Administration of Aqueous Extract of *Apium graveolens* on Oxidative Stress Parameters in Control, HFD and Treated Mice

Catalase levels were measured in the liver, heart, kidney and brain, and the results of the HFD and oral supplementation with an aqueous extract of *Apium graveolens* are reported in Table 6 and Figure 7.

*Apium graveolens* aqueous extract increased catalase activity at doses of 50, 100 and 200 mg/kg in a dose-dependent manner, with the highest increase seen in the liver ($p = 0.0459$), heart ($p < 0.0001$), and kidney ($p = 0.0002$), with the lowest increase seen in the brain ($p = 0.3839$), at the 200 mg/kg dose (Figure 7). Mice that were fed an HFD had considerably lower catalase activity in their organs compared to the control group. This was seen in the liver ($p = 0.0001$), heart ($p = 0.0037$), kidney ($p < 0.0001$), and brain ($p < 0.0001$). Catalase activity was found to increase after 50, 100 and 200mg/kg of *Apium graveolens* aqueous extract were orally administered. The HFD + 200 mg/kg treatment group showed significantly higher levels of catalase activity in the liver ($p < 0.0001$), heart ($p = 0.0001$), and kidney ($p = 0.0001$), but only a marginally higher level in the brain ($p = 0.0646$), compared to the HFD control group. The HFD + 200 mg/kg group, on the other hand, showed significant increases in catalase activity in the liver ($p = 0.0244$), heart ($p < 0.0001$), and brain ($p < 0.0001$) while restoring the catalase activity

**Table 6.** Effect of HFD and oral administration of *Apium graveolens* on oxidative stress markers in the liver, heart, brain and kidney in *Swiss* albino mice for 12 weeks. (Catalase, GSH and MDA = Malonaldehyde)

| Organs | Parameters | Control | 50 mg/kg bw | 100 mg/kg bw | 200 mg/kg bw | HFD | HFD + 50 mg/kg bw | HFD + 100 mg/kg bw | HFD + 200 mg/kg bw |
|---|---|---|---|---|---|---|---|---|---|
| LIVER | Catalase (U/mg protein) | 3.74 ±0.5 | 4.03 ± 0.2 | 3.13 ± 0.08 | 4.36 ± 0.07 | 2.21 ± 0.04 | 4.27 ± 0.1 | 4.45 ± 0.07 | 5.58 ± 0.1 |
| | GSH (µM/mg protein) | 0.74 ± 0.06 | 0.81 ± 0.02 | 0.82 ± 0.06 | 1.02 ± 0.04 | 0.61 ± 0.05 | 0.99 ± 0.04 | 0.82 ± 0.06 | 0.87 ± 0.1 |
| | MDA [µM] | 0.73 ± 0.09 | 0.77 ± 0.13 | 0.53 ± 0.12 | 0.40 ± 0.08 | 1.14 ± 0.07 | 0.59 ± 0.13 | 0.80 ± 0.04 | 0.71 ± 0.14 |
| HEART | Catalase (U/mg protein) | 10.05 ± 0.33 | 10.7 ± 0.14 | 12.3 ± 0.35 | 12.7 ± 0.18 | 7.45 ± 0.25 | 7.55 ± 1.0 | 8.90 ± 0.3 | 12.7 ± 0.13 |
| | GSH (µM/mg protein) | 2.66 ± 0.68 | 3.05 ± 0.51 | 4.46 ± 0.28 | 4.46 ± 0.21 | 0.73 ± 0.06 | 1.45 ± 0.14 | 1.91 ± 0.39 | 3.10 ± 0.26 |
| | MDA [µM] | 1.04 ± 0.07 | 0.91 ± 0.06 | 0.89 ± 0.06 | 0.89 ± 0.09 | 1.32 ± 0.09 | 1.33 ± 0.12 | 1.31 ± 0.12 | 1.04 ± 0.10 |
| BRAIN | Catalase (U/mg protein) | 1.41 ± 0.06 | 1.15 ± 0.10 | 1.30 ± 0.01 | 1.39 ± 004 | 0.71 ± 0.02 | 0.70 ± 0.08 | 0.72 ± 0.05 | 0.82 ± 0.04 |
| | GSH (µM/mg protein) | 0.70 ± 0.02 | 0.70 ± 0.02 | 0.75 ± 0.06 | 0.77 ± 0.03 | 0.61 ± 0.03 | 0.95 ± 0.08 | 1.02 ± 0.08 | 0.95 ± 0.01 |
| | MDA [µM] | 0.04 ± 0.009 | 0.02 ± 0.01 | 0.03 ± 0.01 | 0.02 ± 0.007 | 0.13 ± 0.007 | 0.12 ± 0.17 | 0.11 ± 0.01 | 0.103 ± 0.01 |
| KIDNEY | Catalase (U/mg protein) | 2.45± 0.196 | 2.47 ± 0.208 | 3.13 ± 0.139 | 3.75 ± 0.109 | 0.90 ± 0.012 | 1.13 ± 0.05 | 1.7 ± 0.083 | 2.09± 0.116 |
| | GSH (µM/mg protein) | 0.409 ± 0.01 | 0.43 ± 0.03 | 0.51 ± 0.04 | 0.48 ± 0.02 | 0.36 ± 0.01 | 0.47 ± 0.04 | 0.60 ± 0.02 | 0.64 ± 0.02 |
| | MDA [µM] | 1.15 ± 0.01 | 1.15 ± 0.008 | 1.06 ± 0.02 | 0.85 ± 0.03 | 1.12 ± 0.01 | 1.11 ± 0.01 | 1.09 ± 0.009 | 1.04 ± 0.01 |

The data are expressed as mean ± SEM (n = number of animals in each group = 4). GSH = Glutathione, MDA= Malondialdehyde.

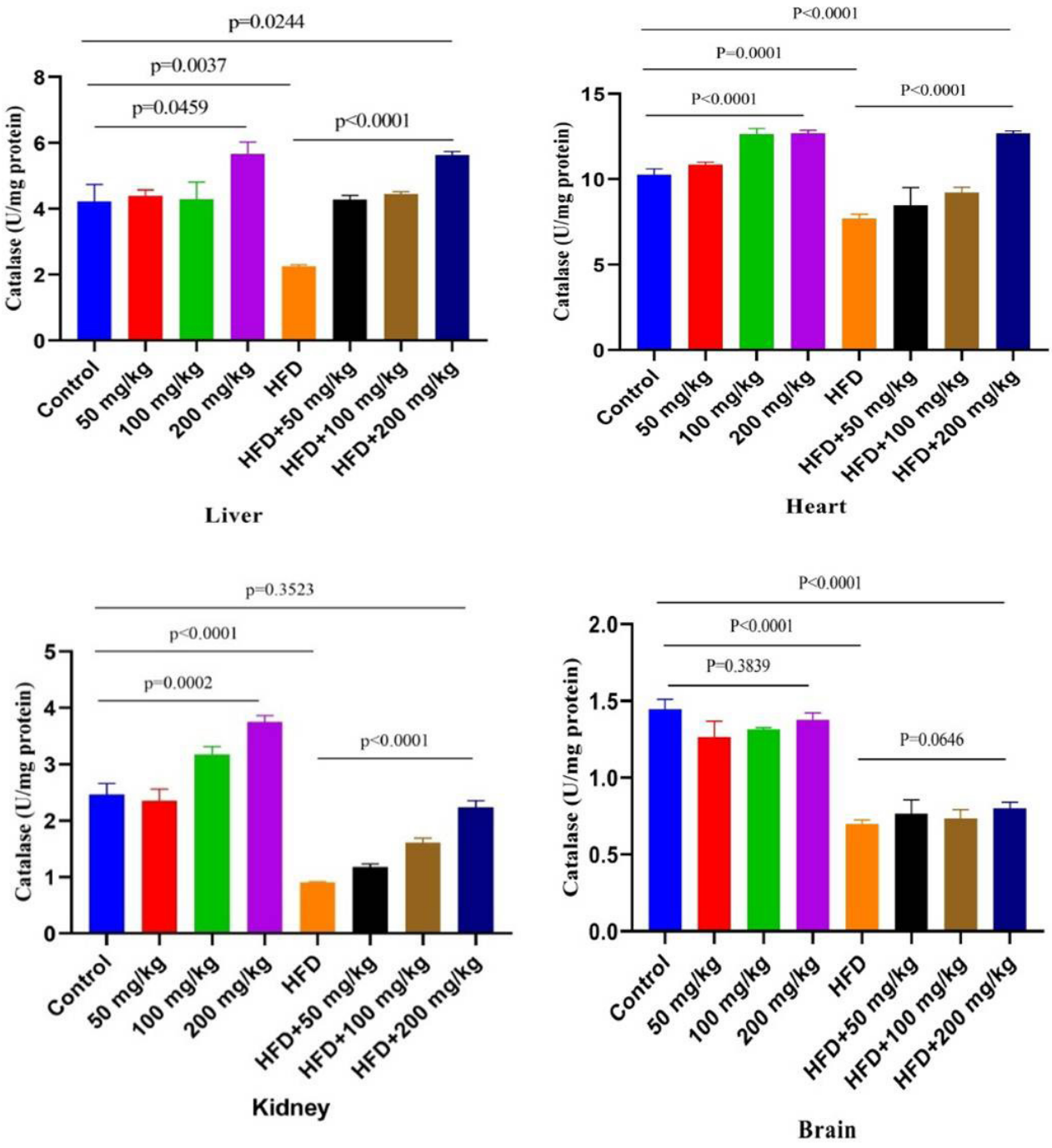



**Figure 7.** The effects of HFD and oral supplementation of *Apium graveolens* extract on catalase activity in the heart, liver, brain and kidney in *Swiss* albino mice for 12 weeks.

to roughly normal level in the kidney (p = 0.3523), as compared to the control group (Figure 7).

The effects of an HFD and oral supplementation with an aqueous extract of *Apium graveolens* on oxidative stress markers in the heart, liver, kidneys and brain are reported in Table 6 and Figure 8.

*Apium graveolens* aqueous extract was given at 50, 100 and 200 mg/kg to increase GSH activity; however, significant increases in GSH activity were only seen when compared to the control group in the liver (p = 0.0044), heart (p = 0.0292), and kidney (p = 0.0509), but not in the brain (p = 0.2772). Liver (p = 0.0022), heart (p = 0.0166)

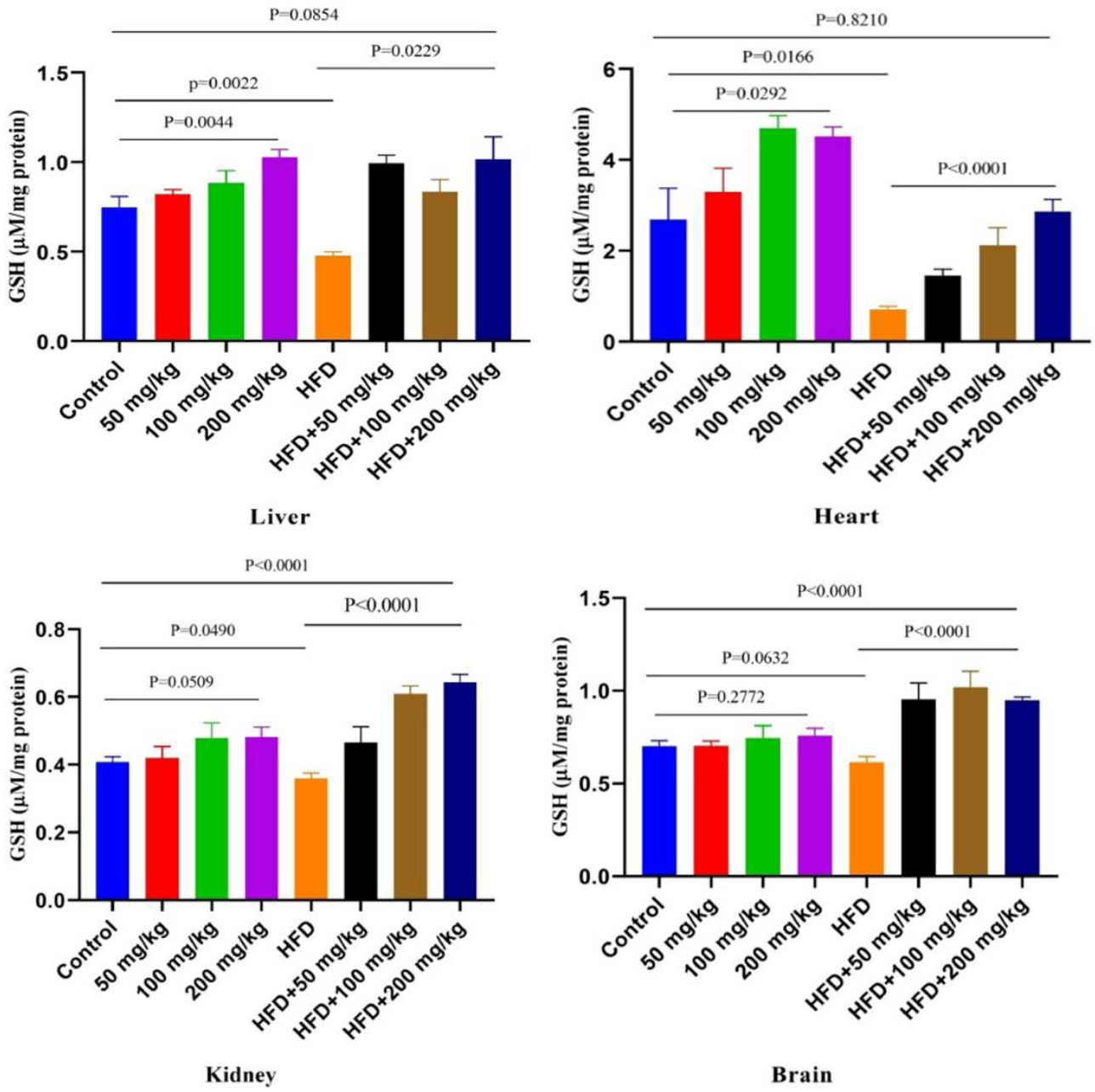



**Figure 8.** The effects of HFD and oral supplementation of *Apium graveolens* extract on GSH activity in the heart, liver, brain and kidney in *Swiss* albino mice for 12 weeks.

and kidney ($p = 0.0490$). GSH activity was considerably lowered in HFD-fed mice compared to the control group; however, brain ($p = 0.0632$) GSH activity was not affected (Figure 8). Glutathione S-transferase (GSH) activity was gradually raised after 50, 100 and 200 mg/kg of *Apium graveolens* aqueous extract was orally administered. Glutathione (GSH) activity was significantly higher in the liver ($p = 0.0229$), heart ($p < 0.0001$), kidney ($p < 0.0001$) and brain ($p < 0.0001$) of the HFD + 200 mg/kg group compared to the HFD control group. Similarly, the HFD + 200 mg/kg group showed significantly higher levels of GSH activity in the kidney ($p < 0.0001$), the brain ($p = 0.0001$), the liver ($p = 0.0854$) and the heart ($p = 0.08210$), relative to the control group.

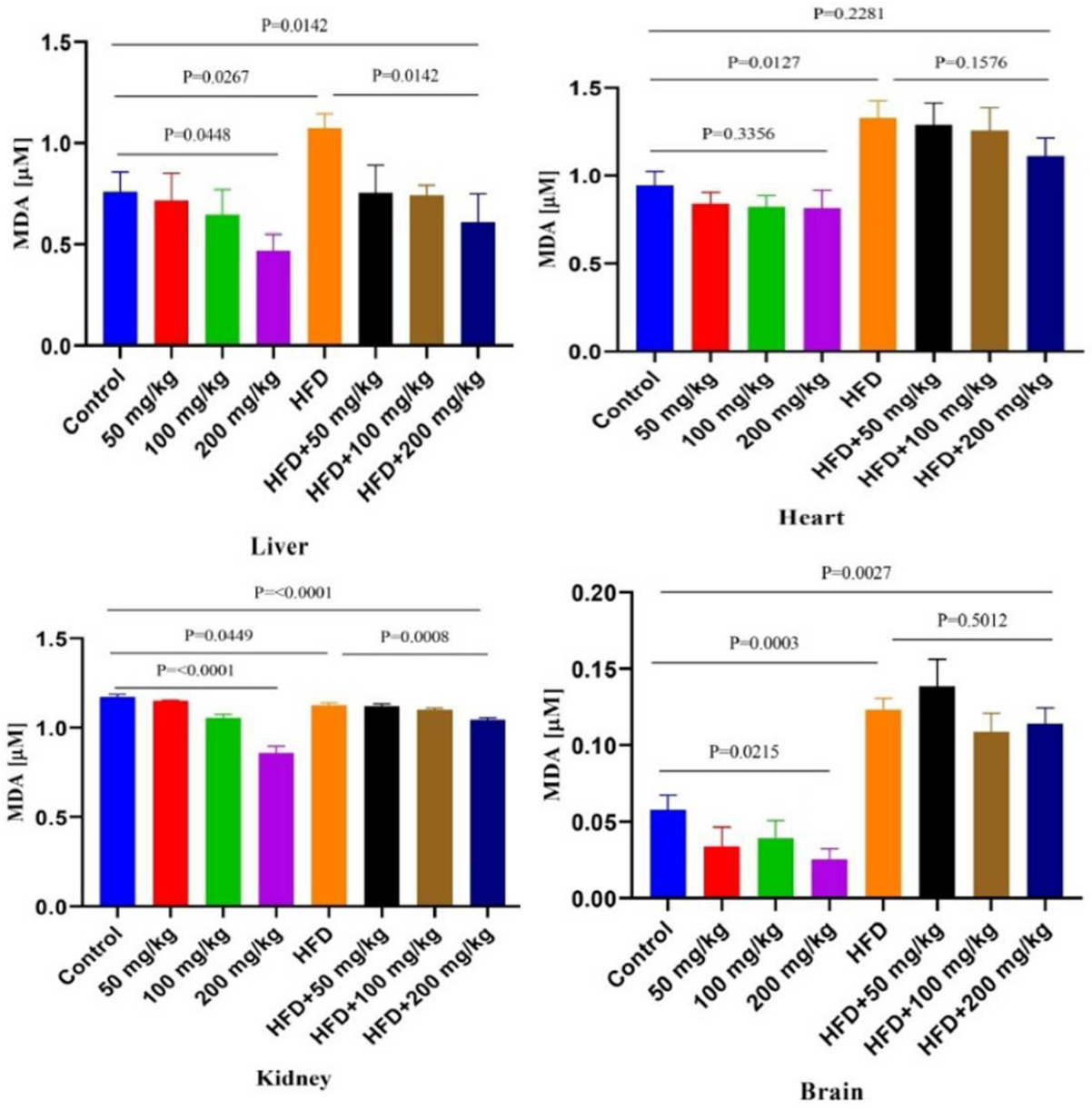



**Figure 9.** The effects of HFD and oral supplementation of *Apium graveolens* extract on MDA level in the heart, liver, brain and kidney in *Swiss* albino mice for 12 weeks.

The effects of HFD and oral supplementation with aqueous extract of *Apium graveolens* on MDA levels in the heart, liver, kidneys and brain are summarized in Table 6 and Figure 9.

Doses of 50, 100 and 200 mg/kg of *Apium graveolens* aqueous extract all had a modest effect on MDA levels compared to the control group, but the effect was statistically significant in the liver ($p = 0.0448$), kidney ($p < 0.0001$), and brain ($p = 0.0215$), but not the heart ($p = 0.3356$). Compared to control mice, HFD-fed animals had significantly higher levels of MDA in their liver ($p = 0.0267$), heart ($p = 0.0127$), kidney ($p = 0.0449$) and brain ($p = 0.0003$) (Figure 9). *Apium graveolens* aqueous extract at 50, 100 and 200 mg/kg orally lowered levels of Malondialdehyde (MDA) over time. Liver MDA levels in the HFD + 200 mg/kg group were significantly lower

than those in the HFD control group (p = 0.0142), kidney MDA levels were lower (p = 0.0008), and heart MDA levels were lower (p = 0.1576) and brain MDA levels were lower (p = 0.5012) as compared to the HFD control group. Similarly, the MDA levels in the liver (p = 0.0142), kidney (p < 0.0001) and brain (p = 0.0027) of the HFD + 200 mg/kg group were significantly lower than those in the control group, but the MDA level in the heart was maintained at roughly normal (p = 0.2281) (Figure 9).

## 7.6 Effect of Oral Administration of Aqueous Extract of *Apium graveolens* on Cardiac Inflammatory Biomarkers mRNA Expression in Control, HFD and Treated Mice

Cardiac inflammatory biomarker mRNA expression was shown to be affected by both HFD and oral administration of *Apium graveolens* extract in Figure 10. The mRNA

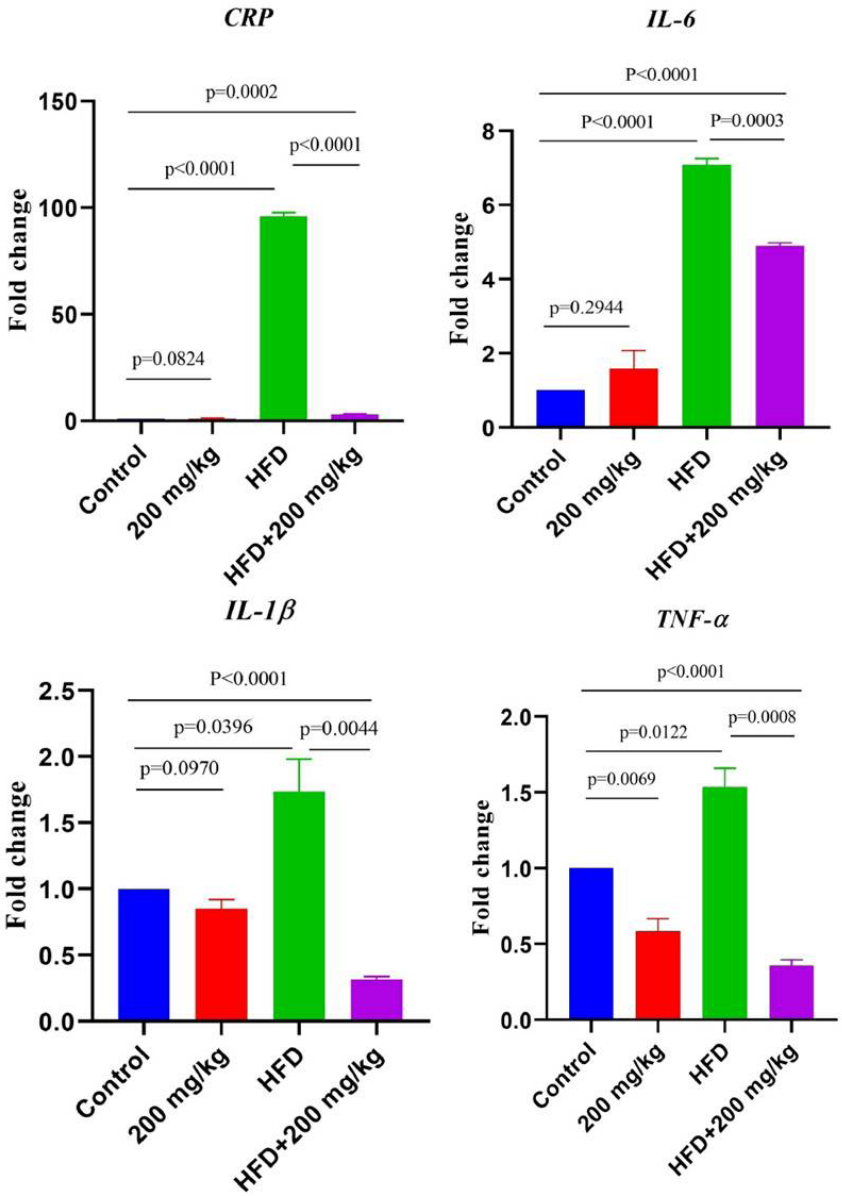
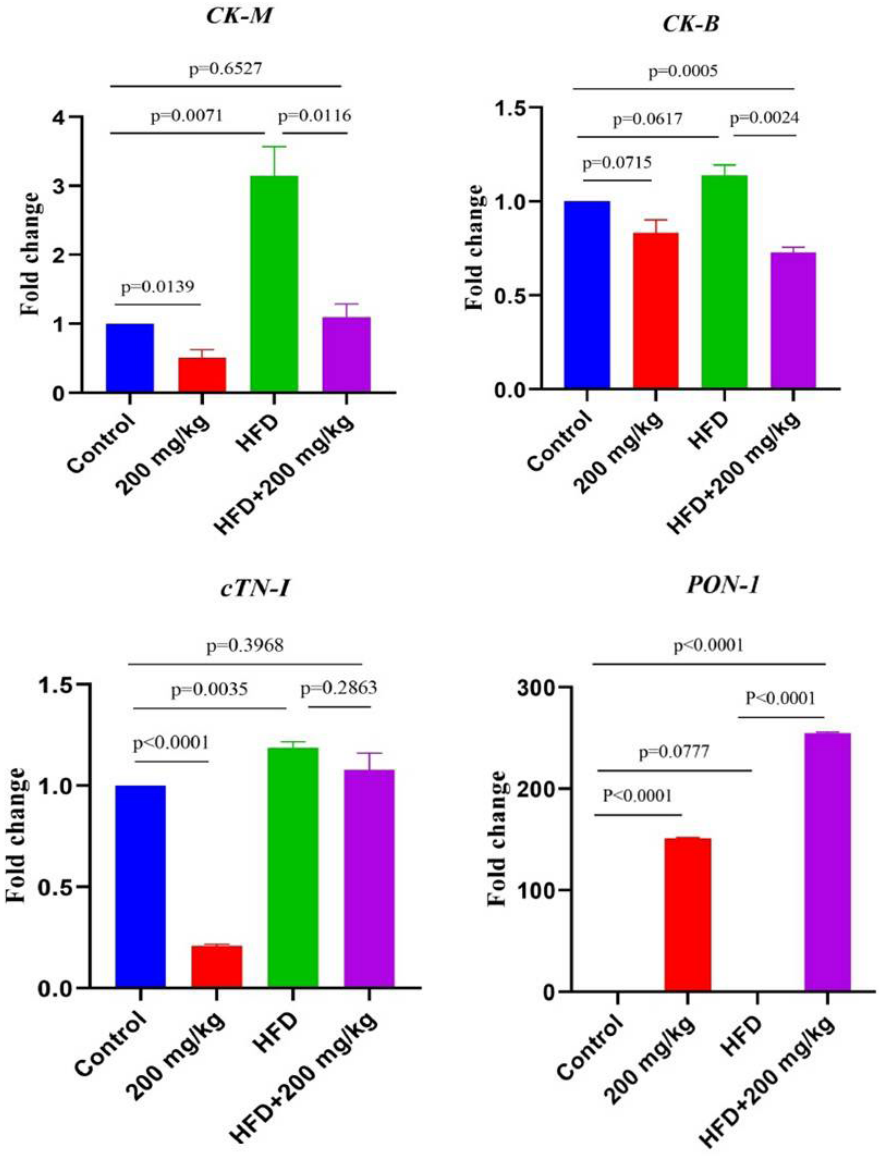



**Figure 10.** Effects of HFD and oral administration of *Apium graveolens* extract on cardiac inflammatory biomarker mRNA expression in *Swiss* albino mice for 12 weeks.

expression of TNF-α (p = 0.0069) and interleukin-1 (p = 0.0970) was down-regulated after oral administration of the aqueous extract at a dose of 200 mg/kg, but changes in C-reactive protein (p = 0.0824) and interleukin-6 (p = 0.2944) were not statistically significant (Figure 10). Compared to control mice, HFD-fed mice had significantly higher mRNA levels of CRP (p < 0.0001), IL-6 (p < 0.0001), IL-1β (p = 0.0396), and TNF-α (p = 0.0122). The mRNA expression of CRP (p < 0.0001), IL-6 (p = 0.0003), IL-1β (p = 0.0044), and TNF-α (p = 0.0008) were down-regulated in the HFD group after oral administration of 200 mg/kg compared to the HFD control group (Figure 10). Comparable changes in CRP (p < 0.0001), IL-6 (p < 0.0001), TNF-α (p = 0.000), and IL-1β (p = 0.0001) mRNA expression were found in the HFD + 200 mg/kg treatment group compared to the control group (Figure 10).

## 7.7 Effect of Oral Administration of Aqueous Extract of *Apium graveolens* on Cardiac CVD Biomarkers mRNA Expression in Control, HFD and Treated Mice

The results of *Apium graveolens* extract and high-fat diet on CVD biomarker mRNA expression are reported in Figure 11.



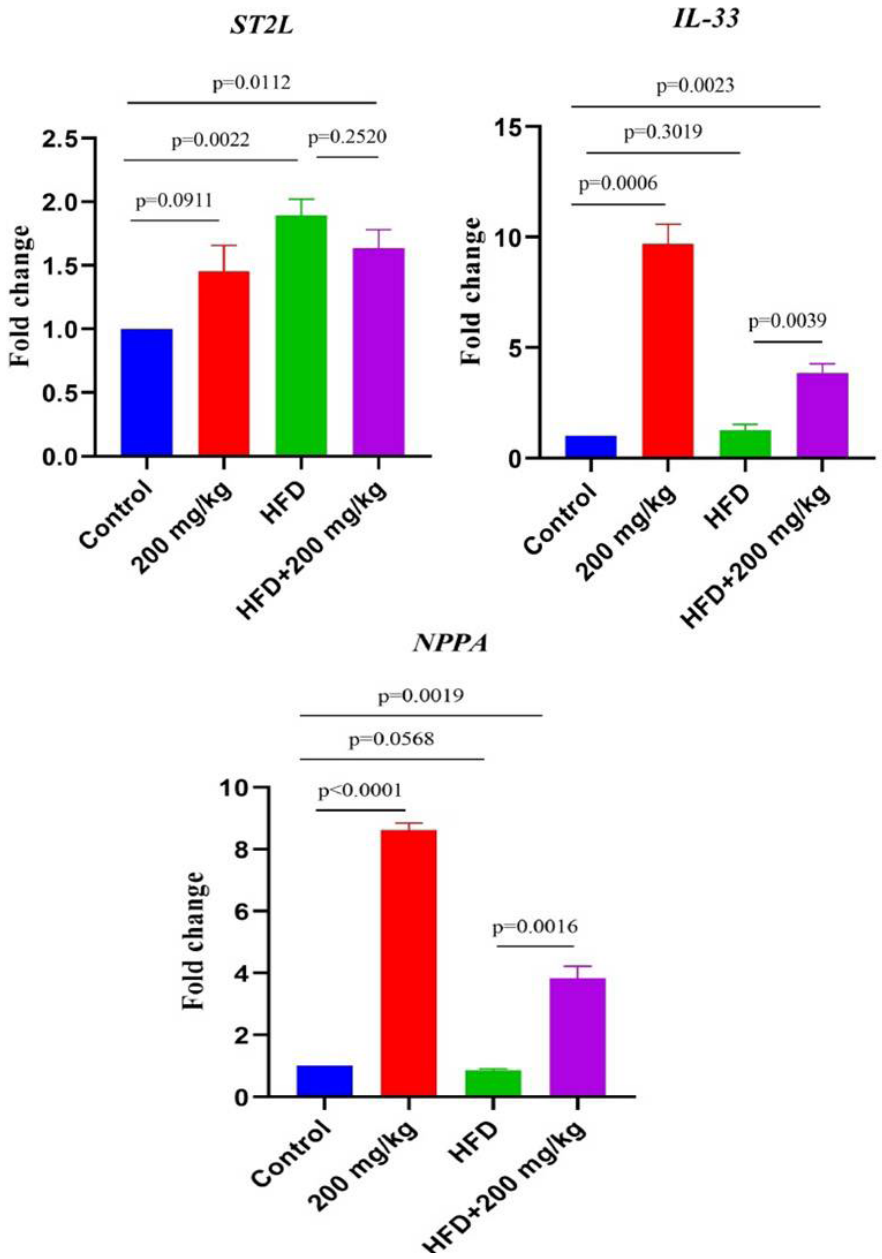



**Figure 11.** Effects of HFD and oral administration of *Apium graveolens* extract on cardiac CVD biomarker mRNA expression in *Swiss* albino mice for 12 weeks.

The mRNA expression of CK-M ($p = 0.0139$), CK-B ($p = 0.0715$), and CTN-I ($p < 0.0001$) was down-regulated after oral administration of the aqueous extract at a dose of 200 mg/kg, while that of PON-1 ($p = 0.0001$), ST2L ($p = 0.0911$), IL-33 ($p = 0.0006$), and NPPA ($p = 0.0001$) was up-regulated (Figure 11). Compared to control mice, HFD-fed mice showed down-regulation of PON-1 ($p = 0.0777$) and NPPA ($p = 0.0568$) and up-regulation of CK-M ($p = 0.0071$), CK-B ($p = 0.0617$), CTN-I ($p = 0.0035$), and ST2L ($p = 0.0022$) mRNA expressions (Figure 11). When given orally to the HFD group at a dose of 200 mg/kg, mRNA expression for CK-M ($p = 0.0116$), CK-B ($p = 0.0024$), CTN-I ($p = 0.2863$), and ST2L ($p = 0.2520$) was reduced, whereas expression for PON-1 ($p<0.0001$), IL-33 ($p = 0.0039$), and NPPA ($p = 0.0016$) was increased. Similarly, the mRNA expression of CK-B

(p = 0.0005) and ST2L (p = 0.0112) was considerably lower in the HFD + 200 mg/kg treated group compared to the control group, although the mRNA expression of CK-M (p = 0.6527) and CTN-I (p = 0.3968) was not statistically different (Figure 11).

### 7.8 Effect of Oral Administration of Aqueous Extract of *Apium graveolens* on Cardiac Proteins in Control, HFD and Treated Mice

The effects of oral administration of *Apium graveolens* extract on Atria Natriuretic Peptides (ANP) in the heart, serum, and kidney were summarized in Table 8 and Figure 12. When compared to the control group, kidney ANP levels increased significantly (p = 0.0149) after oral administration of the aqueous extract at a dose of 200 mg/kg; nevertheless, heart ANP levels increased insignificantly (p = 0.5363), and serum ANP levels increased insignificantly (p = 0.5204). The ANP levels in the hearts (p = 0.0654), serum (p = 0.3272), and kidneys (p = 0.08859) of HFD-fed mice were not statistically different from those in the control group (Figure 12). Oral treatment of 200 mg/kg to the HFD group (HFD + 200 mg/kg) resulted in a substantial rise in ANP level in the heart (p = 0.0018) but had no effect on ANP levels in the serum (p = 0.3576) or the kidneys (p = 0.3123) as compared to the HFD control group. ANP levels were found to be significantly higher in the hearts of the HFD + 200 mg/kg treated group compared to the controls (p = 0.0176), whereas serum (p = 0.9262) and kidney (p = 0.6536) ANP levels were not significantly different (Figure 12).

*Apium graveolens* extract's effects on cardiac troponin-I (CTN-I) in the heart and serum were summarized in Table 7 and Figure 13. Heart CTN-I levels were not significantly lower (p = 0.2066), and serum CTN-I levels were not significantly different (p = 0.1215) after oral administration of the aqueous extract at a dose of 200 mg/kg compared to the control group (Figure 13). The CTN-I level in the hearts of HFD-fed mice was considerably (p = 0.0164) and non-significantly (p = 0.3984) higher than in the serum of control animals. Similarly, oral treatment of 200 mg/kg to the HFD group (HFD + 200 mg/kg) resulted in a significant drop in cardiac CTN-I levels (p = 0.0518) and a non-significant decrease in serum levels (p = 0.5160) as compared to the HFD control group. CTN-I levels in the heart (p = 0.2587) and serum (p = 0.9608) were not significantly different between the HFD+ 200 mg/kg group and the control group (Figure 13).

## 8. Discussion

We compared the effects of an HFD on CVD biomarkers and risk factors in male BALB/c mice with and without celery extract treatment for up to 12 weeks in the control, HFD, and celery treatment groups. Changes in an animal's body or organ mass are usually used as a reliable predictor of its general health. Each animal's weight was checked four times: once before acclimatization, once before the experiment commenced, every week throughout treatment, and once at the conclusion (12 weeks). Organ weights were also measured after the treatment period. Table 2 and Figure 1 show the weekly weight changes for all groups. There was a large disparity in the animals' body weights across the different groups. Weight growth was seen

**Table 7.** The effects of oral supplementation of *Apium graveolens* extract on ANP and CTN-I in the heart, serum and kidney in *Swiss* albino mice for 12 weeks

| Organs | Parameters | Control | 200 mg/kg bw | HFD | HFD + 200 mg/kg bw |
|---|---|---|---|---|---|
| HEART | ANP [ng/ml] | 0.808 ± 0.09 | 0.922 ± 0.08 | 0.487 ± 0.11 | 1.25 ± 0.05 |
| | CTN-I [ng/ml] | 0.23 ± 0.02 | 0.177 ± 0.02 | 0.315 ± 0.01 | 0.253 ± 0.01 |
| SERUM | ANP [pg/ml] | 0.267 ± 0.03 | 0.317 ± 0.02 | 0.23 ± 0.009 | 0.28 ± 0.04 |
| | CTN-I [ng/ml] | 0.021 ± 0.001 | 0.016 ± 0.001 | 0.025 ± 0.003 | 0.018 ± 0.004 |
| KIDNEY | ANP [ng/ml] | 0.295 ± 0.03 | 0.46 ± 0.02 | 0.212 ± 0.02 | 0.274 ± 0.18 |

The data are expressed as mean ± SEM (n = number of animals in each group = 4). ANP = Atrial natriuretic peptide, cTN-I = Cardiac troponin

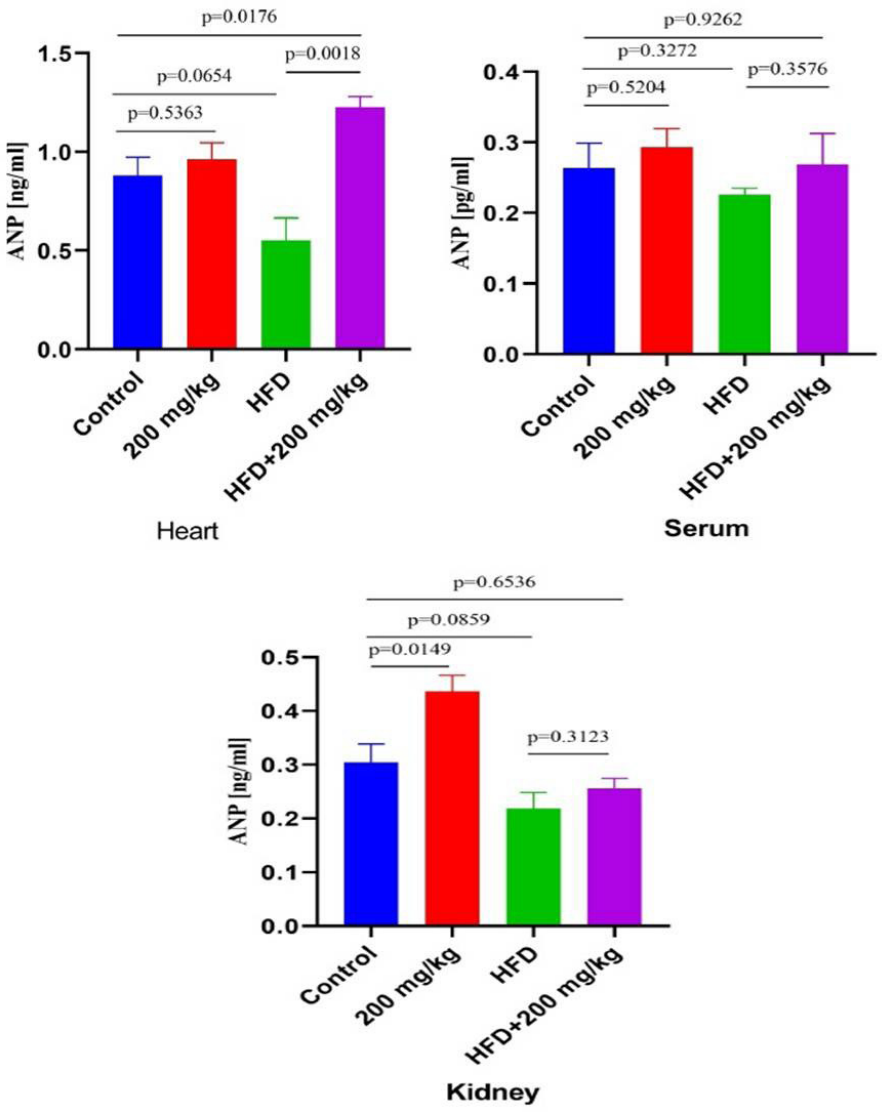



**Figure 12.** The effects of HFD and oral supplementation of *Apium graveolens* extract on ANP = Atria natriuretic peptides in the heart, serum, and kidney in *Swiss* albino mice for 12 weeks.

in the control group throughout treatment, while low-dose oral administration of *Apium graveolens* aqueous extract resulted in minimal weight gain, and high-dose therapy resulted in substantial weight loss (Figure 1). It was also shown that mice fed the HFD acquired considerably more weight than those fed the control diet. Furthermore, it has been documented that cholesterol-rich diet-fed animals were significantly overweight[58,59]. In addition, it has been shown that HFD reduces satiety, leading to overeating and weight gain due to decreased leptin secretion from adipose tissue. It was also shown that rats given an HFD had lower levels of leptin secretion[65]. Low- and high-dose oral administration of the aqueous extracts from the HFD groups led to similar rates of weight loss. Animals given HFD + 200 mg/kg had surprisingly stable body weights when compared before and after treatment (Figure 2). HFD and celery extract treatment had no significant effect on the weight of vital organs such as the kidney, heart, brain, and spleen. In comparison to the control and HFD control groups, the 200 mg/kg group revealed a substantial reduction in liver weight, while the HFD group showed a significant rise in liver weight (Figure 3). HFDs increase liver weight due to increased fat consumption, which may lead to non-alcoholic fatty liver disease[66].

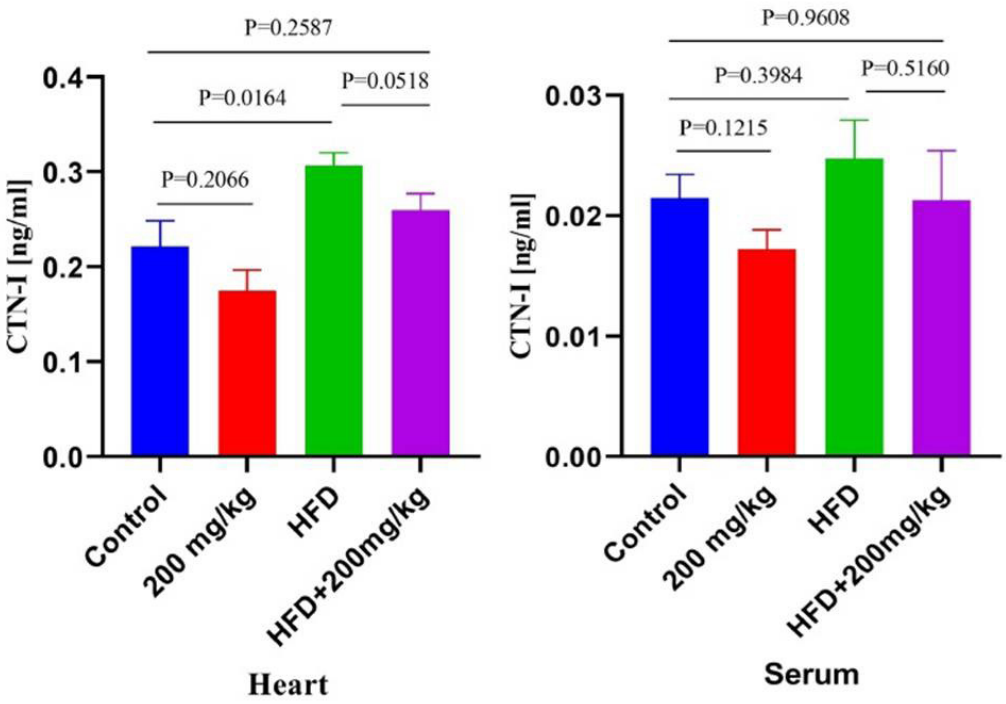



**Figure 13.** The effects of HFD and oral supplementation of *Apium graveolens* extract on CTN-I = cardiac troponin-I in the heart and serum in *Swiss* albino mice for 12 weeks.

Liver weight increased in the HFD group and decreases after they received oral treatment at a dosage of 200 mg/kg (Figure 3). Given these results, we postulated that *Apium graveolens* medicine would be useful for the treatment and/or prevention of fatty liver. One difficulty in reading organ weight data is figuring out if a chemical is having an immediate effect on an organ or if it's only affecting the organ indirectly through weight gain or loss. The "relative organ weight" of each animal is obtained by dividing the sum of the weight of its organs by its total body weight, which allows us to compare the severity of the problem between different species. Results from this study suggest that both the HFD and the *Apium graveolens* leaf extract have direct impacts on liver weight by interfering with the liver's lipid metabolism while having very little effect on the heart, kidney, brain, and spleen when compared to the control and HFD controls (Figure 4). Celery has been shown to reduce body and liver weight, which may be attributable to the presence of phytochemicals such as polyphenols, saponins, and others[67]. These phytochemicals may have inhibited lipogenesis by interfering with nutritional absorption in the intestine, thereby reducing the body's reliance on dietary fat. Herbal remedies (such as herbal formulations, crude extracts, and pure bioactive components from medicinal plants) have been shown to decrease hepatic lipogenesis[68]. Inhibiting Phosphodiesterase (PDE) or boosting thermogenesis via Uncoupling Proteins (UCP1, 2, or 3) can also be used to promote lipolysis, either directly or indirectly through an Angiotensin-Converting Enzyme (ACE)[68,69]. The findings suggest that *Apium graveolens* aqueous extract might aid in weight reduction and fatty liver.

Dyslipidemia (hypercholesterolemia and hypertriglyceridemia) was indicated by elevations in lipid parameters, all of which increase the risk of atherosclerosis and CVDs[70]. Serum cholesterol, triglycerides, LDL-C, and HDL-C levels were all substantially ($P < 0.05$) higher in the HFD group compared to the control group, indicating the development of dyslipidemia (Figure 5). After 12 weeks of low- or high-dose oral administration of *Apium graveolens* aqueous extract, serum levels of cholesterol, triglycerides, LDC-C, and VLDL-C were all lowered compared to the control group, while HDL-C levels were elevated (Figure 5). When compared to the HFD control group, *Apium graveolens* dramatically reduced blood levels of cholesterol, triglycerides, LDL-

C, and VLDL-C while increasing serum levels of HDL-C (Figure 5). *Apium graveolens* aqueous extracts reversed the dyslipidemic lipid profile observed in HFD-treated mice, likely via inhibiting HMG-CoA reductase like atorvastatin[71,72]. The extract contains saponins, which may help with dyslipidemia treatment. Specifically, saponin-rich extracts have been demonstrated to inhibit pancreatic lipase, a key enzyme in lipid metabolism[73]. Flavonoids are also known to reduce atherogenic LDL-C oxidation[74]. The ability of *Apium graveolens* aqueous extracts to increase HDL may contribute to the plant's anti-atherosclerosis characteristics because HDL-C possesses anti-inflammatory and antioxidant properties[75].

There is a correlation between elevated levels of lipid markers, such as AI, CRR, and AC, and an increased likelihood of developing cardiovascular illnesses[12]. The lipid-lowering effects of *Apium graveolens* aqueous extracts were further confirmed by measuring AI, CRR, and AC, which were all shown to be substantially ($p < 0.05$) lower in the celery-treated group, especially at the dose of 200mg/kg. Significant ($p < 0.05$) elevations in AI, CRR, and AC were seen between HFD-fed mice and normal control animals (Figure 5). Treatment of HFD-fed animals with modest dosages of *Apium graveolens* aqueous extracts resulted in a significant reduction ($p < 0.05$) when compared to the HFD control group. A similar reduction in AI and a very significant ($p < 0.05$) reduction in CRR and AC were seen in mice given the HFD at a dosage of 200 mg/kg compared to the normal control group (Figure 5). *Apium graveolens* aqueous extract was shown to reduce AI, CRR and AC values across all treatment groups, suggesting it may be useful in avoiding atherosclerosis, coronary artery disease, and CVDs.

Serum levels of liver-derived enzymes, nonenzymatic proteins, and metabolites metabolized by the liver are commonly measured in clinical practice (colloquially referred to as liver function tests or LFTs). Subtle LFT abnormalities are common in patients at risk for or already suffering from CVDs. Recent research suggests that certain liver enzymes have been implicated with MetS, hypertension, and CVDs[76]. Increase blood glucose levels are a risk factor for CVDs even in those who, on the surface, appear to be healthy, whether or not they have diabetes[77]. Mice given an HFD for 12 weeks showed increases in blood glucose levels (Figure 6). There is some evidence to suggest that eating a lot of saturated fat and eating a lot of total fat both contribute to weight gain, which in turn increases the risk of developing diabetes[78]. *Apium graveolens* aqueous extract, taken orally for 12 weeks at low or high doses, significantly reduced blood glucose and prevented blood glucose rise compared to the normal control and HFD control groups (Figure 6). In conclusion, *Apium graveolens* showed promise as a hypoglycemic agent. *Apium graveolens* has been hypothesized to work because of its high polyphenol content[67]. Carbohydrate metabolism may be disrupted by polyphenols like flavonoids, phenolic acids and others by decreasing digestion and absorption of carbohydrates, increasing the production of insulin by pancreatic cells, decreasing gluconeogenesis from the liver, activating insulin receptors, and increasing glucose uptake in muscle and fat[79]. Similarly, mice given the HFD had significantly elevated blood levels of SGPT, SGOT, gamma-glutamyl transferase, direct Bilirubin, uric acid and LDH compared to the control group, except for total Bilirubin (Figure 6). In addition, oxidative stress caused by HFD has been linked to elevated levels of liver markers[80,81]. Also, it has been demonstrated that food rich in fat and cholesterol leads to an increase in liver weight, fat deposition, inflammation, and fibrosis, as well as an increase in liver enzyme plasma activity[82]. Hepatocellular liver damage, characterized by elevated SGPT and SGOT levels, is thought to result from inflammation and/or injury to liver cells in mice fed an HFD. SGPT and SGOT are two enzymes that are released into the circulation when the liver is damaged[83]. There are several potential molecular mechanisms linking elevated GGT levels to CVDs, including oxidative stress and lifestyle choices[84]. There are two types of bilirubin: Conjugated (direct) and unconjugated (indirect). Total bilirubin is the sum of conjugated and free bilirubin. In healthy people, conjugated bilirubin levels are quite low, and elevated levels point to liver disease. Total bilirubin in the blood is inversely connected to the risk of developing CHD and other CVDs, and higher total bilirubin levels are associated with a protective effect[85]. Our research revealed that HFD-induced liver impairment led to a rose in direct and conjugated bilirubin levels, despite a reduction in total serum bilirubin levels in the HFD-treated animals. Serum UA and LDH levels were similarly elevated in HFD-treated animals (Figure 6). The incidence of CVDs and cardiovascular events is substantially correlated with elevated serum LDH levels. This suggests that serum LDH levels have the potential as a new objective biological marker for the prediction of CVD events[86]. In humans, Uric Acid (UA) is thought to be the result of purine metabolism. Increases in serum UA have been linked with

CVD illness[87]. All parameters were significantly decreased after 12 weeks of oral administration of *Apium graveolens* aqueous extract at low or high dosages in comparison with the normal and HFD control groups (Figure 6). Total Bilirubin levels were increased, while those of uric acid, LDH, uric acid, and direct Bilirubin were lowered, confirming the hepatoprotective and cardioprotective properties of *Apium graveolens*.

Hypercholesterolemic atherogenesis is linked to an increase in the formation of ROS[88]. Mice fed a diet of celery or an HFD diet had their endogenous enzyme levels in their liver, heart, kidneys and brains measured and compared. CAT is mostly found in certain cells where its main function is to catalyze the breakdown of hydrogen peroxide[89]. Hence, the most destructive radicals in vivo, the hydroxyl radical, are blocked from being produced by hydrogen peroxide[90]. Protecting the reduced state and against oxidative stress are both dependent on GSH's presence. To reduce hydrogen peroxide, GSH is converted to GSSG. In response to an increase in GSSG levels, GSH-reductase activity was activated, transforming GSSG into GSH[91]. Due to oxidative stress, the decreased GSH level suggests increased consumption[92]. The lipid peroxidation marker Malondialdehyde (MDA) is produced when oxygenated active species react with fatty acids in membranes[93]. Statistically significant decreases in the activities of CAT and GSH and an elevated MDA level indicated a higher degree of oxidative stress in the HFD-fed mice compared to the mice fed a normal diet in the current analysis (Figures 7, 8 and 9). In comparison to the control and HFD-fed groups, those treated with *Apium graveolens* aqueous extract at either low or high doses showed significantly and dose-dependently higher levels of CAT, GSH, and reduction in MDA level (Figures 7, 8 and 9). This provided conclusive evidence of the anti-oxidant capacity of an aqueous extract of *Apium graveolens* against oxygen free radicals by stimulating antioxidant enzymes and decreasing free radical formation, hydrogen peroxide breakdown, quenching active singlet oxygen, and trapping and quenching radicals[94].

Treating inflammation may offer a unique technique for reducing the risk of sudden cardiac arrest and other cardiovascular crises since inflammation plays a significant role in the genesis, progression, and clinical presentation of CVD[95]. Many inflammatory markers, including CRP, IL-6, IL-1$\beta$, and TNF-α, were significantly up-regulated ($p > 0.05$) in the cardiac tissue of HFD-treated mice compared to the control group in this study (Figure 10). This suggests that consuming high-fat dairy products daily may lead to chronic low-grade inflammation. CRP, IL-6, IL-1$\beta$ and TNF-α mRNA expression was significantly reduced in the *Apium graveolens* aqueous extract-treated group compared to the control group and the HFD control group at a dosage of 200 mg/kg body weight (Figure 10). Research trials have shown that individuals with a history of myocardial infarction and increased CRP levels benefit from IL-1β inhibition with a particular monoclonal antibody (Canakinumab) and that this benefit is related to a decrease in IL-6 levels[96]. Inhibiting the generation of these inflammatory markers demonstrated the anti-inflammatory properties of *Apium graveolens*. Phytocompounds such as tannin, phenolic, flavonoids and related polyphenols may be responsible for their anti-inflammatory effects. Though it has anti-inflammatory qualities, further study is needed to pinpoint the particular mechanism and phytochemical components involved.

In the cardiac tissue of HFD-fed mice, mRNA expression of CVD biomarkers, including ST2L was up, whereas mRNA expression of PON-1 and IL-33 was lowered (Figure 11). Atherosclerosis is more common in people with low PON1 activity. The decrease in IL-33 levels are also linked with a high chance of developing heart failure. The expression of inflammatory markers like TNF-α and IL-6 was correlated with the quantity of sST2 mRNA, as was previously mentioned[97]. As we found that TNF-α and IL-6 mRNA expression levels increased in our study, we hypothesize that this led to an increase in sST2, which then competed with ST2L and inhibited its binding to IL-33 (sST2/IL-33 interaction), thereby reducing the effectiveness of the cardioprotective IL-33/ST2L pathway in HFD-treated mice. In this study, the IL-33/ST2L connection is altered in HFD-treated mice, which may have negative consequences for cardiac health. Nevertheless, PON-1, IL-33, and ST2L expressions were raised after being treated with *Apium graveolens* aqueous extract for 12 weeks (Figure 11). Therefore, mRNA expression levels showed that the IL-33/ST2L link was still active, which may also explain why *Apium graveolens* has cardioprotective effects. Similarly, the mRNA expression of CK-M, CK-B, and CTN-I in heart tissue, as well as the protein concentrations in the heart (CTN-I), serum (CK, and CK-MB), and kidney (ANP) were increased ($p < 0.05$), while the mRNA expression of NPPA and its protein (ANP) level decreased significantly in the HFD group (Figure 11). Consumption of high-fat

dairy products regularly has been linked to increased levels of these markers, which in turn increases the risk of myocardial infarction due to tissue injury/inflammation of the heart muscle (Myocarditis) (MI). Heart attacks are extremely unlikely to occur in people whose blood CTN-I levels are within the normal range; nevertheless, those whose CTN-I mRNA and protein expression in heart tissue is higher than normal are at a greater risk of experiencing MI in the future. Due to decreased NPPA expression and ANP production, mice fed an HFD have higher rates of hypertension and metabolic syndrome[98]. Treatment with 200 mg/kg of *Apium graveolens* aqueous extract for 12 weeks reduced mRNA expression of CK-M, CK-B, and CTN-I, as well as serum CK, CK-MB, and CTN-I, whereas it increased mRNA expression of NPPA in heart tissue and its protein (ANP) level in heart, serum, and kidney compared to the control group (Figures 11 and 12). The fact that these markers dropped significantly in the celery group suggests that *Apium graveolens* has cardioprotective properties. Since ANP promotes lipolysis, which decreases body weight gain and fatty liver (as we've already discussed), prevents oxidative stress, and increases insulin sensitivity, the decrease in serum glucose level in the celery-treated group may be attributable to *Apium graveolens* cardioprotective effects. ANP lowers blood pressure, prevents atherosclerosis, and increases cardiac and vascular vasodilation. The kidney's ability to produce natriuresis and diuresis in response to ANP, together with the relaxation of vascular smooth muscles, allows for more precise regulation of blood volume and pressure[99].

## 9. Conclusion

The conclusions drawn from this investigation indicate a high-fat diet increases body weight and organ weight, notably liver weight. *Apium graveolens* aqueous extracts administered orally lowered body weight and liver weight. Given this data, we postulated that *Apium graveolens* medicine might benefit in the prevention of fatty liver and may promote weight reduction. HFD brought about dyslipidemia (hypercholesterolemia and hypertriglyceridemia). The prevention of dyslipidemia by *Apium graveolens* aqueous extracts indicates the plant›s anti-atherosclerosis potential. HFD elevated serum glucose levels. The treatment with *Apium graveolens* aqueous extract decreased blood glucose significantly and prevented a rise in blood glucose. The results suggested that *Apium graveolens* might be used as an anti-diabetic medication. The increase in liver enzyme markers indicates that a high-fat diet caused liver damage. Yet, *Apium graveolens* aqueous extracts significantly decreased all evaluated parameters, confirming the plant›s hepatoprotective qualities. HFD promotes the generation of ROS species. This buildup causes oxidative stress and redox abnormalities. *Apium graveolens* aqueous extract treatment increases antioxidant enzymes and decreases tissue peroxidation. This conclusively showed the anti-oxidant capability of *Apium graveolens* aqueous extract against oxygen free radicals by increasing antioxidant enzymes and decreasing free radical production. HFD stimulated the mRNA expression of several inflammatory markers. Treatment with *Apium graveolens* aqueous extracts decreased their expression; this shows that *Apium graveolens* has anti-inflammatory properties due to its capacity to suppress the formation of these inflammatory markers. Moreover, HFD enhanced the mRNA expression of several CVD biomarkers. Yet, *Apium graveolens* aqueous extracts significantly reduced the expression of CVD biomarkers, confirming the plant›s cardio-protective properties. As a result, consuming *Apium graveolens* may aid in the prevention of CVDs.

## 10. References

1. Saleem U, Riaz S, Ahmad B, Saleem M. Pharmacological screening of *Trachyspermum Ammi* for antihyperlipidemic activity in Triton X-100 induced hyperlipidemia rat model. Pharmacognosy Res. 2017; 9(1):S34-40. https://doi.org/10.4103/pr.pr_37_17
2. Cardiovascular Disease Foundation [Internet]. What is cardiovascular disease; 2011. World Health Organization (WHO). Cardiovascular diseases (CVDs) [Fact Sheet]. 2021. Available at https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds)
3. Roger VL, Lloyd-Jones D, Berry JD. Heart disease and stroke statistics 2011 update: A report from the American Heart Association. Circulation. 2011; 123:180-209. https://doi.org/10.1161/CIR.0b013e3182009701
4. Thompkinson DK, Bhavana V, Kanika P. Dietary approaches for the management of cardiovascular health- A review. J Food Sci Technol. 2014; 51(10):2318-30. https://doi.org/10.1007/s13197-012-0661-8

5. Olorunnisola OS, Bradley G, Afolayan AJ. Ethnobotanical information on plants used for the management of cardiovascular diseases in Nkonkobe municipality. J Med Plant Res. 2011; 5:4256-60.
6. Brunner H, Cockcroft JR, Deanfield J, Donald A, Ferrannini E, Halcox J, *et al*. Working group on endothelins and endothelial factors of the european society of hypertension. Endothelial function and dysfunction. Part II: Association with cardiovascular risk factors and diseases. A statement by the working group on endothelins and endothelial factors of the European society of hypertension. J Hypertens. 2005; 23:233-46. https://doi.org/10.1097/00004872-200502000-00001
7. Hossain P, Kawar B, El Nahas M. Obesity and diabetes in the developing world - A growing challenge. N Eng J Med. 2007; 356:213-15. https://doi.org/10.1056/NEJMp068177
8. Vogiatzi G, Tousoulis D, Stefanadis C. The role of oxidative stress in atherosclerosis Hellenic. J Cardiol. 2009; 50:402-9. PMID: 19767282.
9. Radhika S, Smila KH, Muthezhilan R. Cardioprotective activity of *Hybanthus enneaspermus* (Linn.) on isoproterenol-induced rats. Ind J Fundam Appl Sci. 2011; 1:90-7.
10. Sheweita SA, Abd El-Gabar M, Bastawy M. Carbon tetrachloride-induced changes in the activity of Phase II drug-metabolizing enzyme in the liver of male rats: Role of antioxidants. Toxicology. 2001; 165:217-24. https://doi.org/10.1016/S0300-483X(01)00429-2
11. Adigun NS, Oladiji AT, Ajiboye TO. The antioxidant and anti-hyperlipidemic activity of hydroethanolic seed extract of *Aframomum melegueta* K. Schum in Triton X-100 induced hyperlipidemic rats. S Afr J Bot. 2006; 105:324-32. https://doi.org/10.1016/j.sajb.2016.03.015
12. Hallenbeck JM, Hansson GK, Becker KJ. Immunology of ischemic vascular disease: Plaque to attack. Trends Immuno. 2005; l(26):550-6. https://doi.org/10.1016/j.it.2005.08.007
13. Mohale DS, Dewani AP, Saoji AN, Khadse CD. Antihyperlipidemic activity of isolated constituents from *Lagenaria siceraria* in albino rats. Int J Green Pharm. 2008; 2:104-7. https://doi.org/10.4103/0973-8258.41181
14. Hadi NR, Mohammad BI, Ajeena IM, Sahib HH. The antiatherosclerotic potential of clopidogrel: Antioxidant and anti-inflammatory approaches. BioMed Res Internt. 2013; 2013:1-10. https://doi.org/10.1155/2013/790263
15. Daniela S, Guido I. Inflammation and cardiovascular diseases: The most recent findings. Int J Mol Sci. 2019; 20:3879. https://doi.org/10.3390/ijms20163879
16. Abdulhamied A, Martin SS, Leucker TM, Michos ED, Blaha MJ, Lowenstein CJ, *et al*. Inflammation and cardiovascular disease: From mechanisms to therapeutics. Am J Phys Chem. 2020; 4:100-30. https://doi.org/10.1016/j.ajpc.2020.100130
17. Vigushin DM, Pepys MB, Hawkins PN. Metabolic and scintigraphic studies of radioiodinated human C-reactive protein in health and disease. J Clin Invest. 1993; 91:1351-7. https://doi.org/10.1172/JCI116336
18. Sattar N, McCarey DW, Capell H, McInnes IB. Explaining how 'high grade' systemic inflammation accelerates vascular risk in rheumatoid arthritis. Circulation. 2003; 108:2957-63. https://doi.org/10.1161/01.CIR.0000099844.31524.05
19. Nakamura M, Yoshida H, Arakawa N, Saitoh S, Satoh M, Hiramori K. Effects of tumor necrosis factor-alpha on basal and stimulated endothelium-dependent vasomotion in human resistance vessel. J Cardiovasc Pharmacol. 2000; 36:487-92. https://doi.org/10.1097/00005344-200010000-00011
20. Yoshizumi M, Perrella MA, Burnett JC, Lee ME. Tumor necrosis factor downregulates an endothelial nitric oxide synthase mRNA by shortening its half-life. Circ Res. 1993; 73(1):205-9. https://doi.org/10.1161/01.RES.73.1.205
21. Hansson GK, Libby P, Tabas I. Inflammation and plaque vulnerability. J Intern Med. 2015; 278:483-93. https://doi.org/10.1111/joim.12406
22. Ridker PM. From C-reactive protein to interleukin-6 to interleukin-1: Moving upstream to identify novel targets for atheroprotection. Circ Res. 2016; 118:145-56. https://doi.org/10.1161/CIRCRESAHA.115.306656
23. Vasan RS. Biomarkers of cardiovascular disease molecular basis and practical considerations. Circulation. 2006; 113(19):2335-62. https://doi.org/10.1161/CIRCULATIONAHA.104.482570
24. Dimarakis I, Joshi V, Hesford W, Asimakopoulos G. 3 - minimized extracorporeal circulation: Physiology and pathophysiology. T. Gourlay, S. Gunaydin, editors. In: Wood head publishing series in biomaterials, minimized cardiopulmonary bypass techniques and technologies. Wood Head Publishing; 2012. p. 35-44, https://doi.org/10.1533/9780857096029.1.35
25. Brewster LM. Creatine kinase, energy reserve, and hypertension: From bench to bedside. Ann Transl Med. 2018; 6(15):292. https://doi.org/10.21037/atm.2018.07.15

26. Karamat FA, Oudman I, Haan YC, van Kuilenburg ABP, Leen R, *et al*. Creatine kinase inhibition lowers systemic arterial blood pressure in spontaneously hypertensive rats: A randomized controlled trial. J Hypertens. 2016; 34:2418-26. https://doi.org/10.1097/HJH.0000000000001090
27. Makoto W, Tomonori O, Yoshihiro K, Aya H, Akira O. Elevated serum creatine kinase predicts first-ever myocardial infarction: A 12-year population-based cohort study in Japan, the Suita study. *Int J* Epidemiol. 2009; 38:1571-9. https://doi.org/10.1093/ije/dyp212
28. Garg P, Morris P, Fazlanie AL, Vijayan S, Dancso B, Dastidar AG, *et al*. Cardiac biomarkers of the acute coronary syndrome: From history to high-sensitivity cardiac troponin. Intern Emerg Med. 2017; 12(2):147-55. https://doi.org/10.1007/s11739-017-1612-1
29. Keller T, Zeller T, Peetz D, Tzikas S, Roth A, Czyz E, Bickel C, *et al*. Sensitive troponin I assay in early diagnosis of acute myocardial infarction. N Engl J Med. 2009; 361(9):868-77. https://doi.org/10.1056/NEJMoa0903515
30. Harrison T, Longo D, Kasper D, Jameson J, Fauci A, Hauser S, *et al*. Harrison's principles of internal medicine. New York: McGraw- Hill; 2012.
31. Hassan AK, Bergheanu SC, Hasan-Ali H, Liem SS, van der Laarse A, Wolterbeek R, *et al*. Usefulness of peak troponin-T to predict infarct size and long-term outcome in patients with first acute myocardial infarction after primary percutaneous coronary intervention. Am J Cardiol. 2009; 103(6):779-84. https://doi.org/10.1016/j.amjcard.2008.11.031
32. Sze J, Mooney J, Barzi F, Hillis GS, Chow CK. Cardiac troponin and its relationship to cardiovascular outcomes in community populations- A systematic review and meta-analysis. Heart, Lung and Cir. 2016; 25:217-28. https://doi.org/10.1016/j.hlc.2015.09.001
33. Potter LR, Abbey-Hosch S, Dickey DM. Natriuretic peptides, their receptors, and cyclic guanosine monophosphate-dependent signaling functions. Endocr Rev. 2006; 27:47-72. https://doi.org/10.1210/er.2005-0014
34. Wu Q, Xu-Cai YO, Chen S, Wang W. Corin: New insights into the natriuretic peptide system. Kidney Int. 2009; 75:142-6. https://doi.org/10.1038/ki.2008.418
35. Levis ER, Gardner DG, Samson WK. Natriuretic peptides. N Engl J Med. 1998; 339:321-8. https://doi.org/10.1056/NEJM199807303390507
36. Theilig F, Wu Q. ANP-induced signaling cascade and its implications in renal pathophysiology. Am J Physiol Renal Physiol. 2015; 308(10):F1047-55. https://doi.org/10.1152/ajprenal.00164.2014
37. Zeidel ML. Renal actions of atrial natriuretic peptide: Regulation of collecting duct sodium and water transport. Annu Rev Physiol. 1990; 52:747-59. https://doi.org/10.1146/annurev.ph.52.030190.003531
38. Chow SL, Maisel AS, Anand I, Bozkurt B, de Boer RA, Felker GM, Fonarow GC, Greenberg B, Januzzi JL Jr, Kiernan MS, Liu PP, Wang TJ, Yancy CW, Zile MR. American Heart Association Clinical Pharmacology Committee of the Council on Clinical Cardiology; Council on Basic Cardiovascular Sciences; Council on Cardiovascular Disease in the Young; Council on Cardiovascular and Stroke Nursing; Council on Cardiopulmonary, Critical Care, Perioperative and Resuscitation; Council on Epidemiology and Prevention; Council on Functional Genomics and Translational Biology; and Council on Quality of Care and Outcomes Research. Role of Biomarkers for the Prevention, Assessment, and Management of Heart Failure: A Scientific Statement From the American Heart Association. Circulation. 2017; 135(22):e1054-91. https://doi.org/10.1161/CIR.0000000000000490
39. Biaggi P, Ammann C, Imperiali M, Hammerer-Lercher A, Breidthardt T, Müller C, *et al*. Soluble ST2- A new biomarker in heart failure. Cardiovasc Med. 2019; 22:w02008. https://doi.org/10.4414/cvm.2019.02008
40. Miller AM, Xu D, Asquith DL, Denby L, Li Y, Sattar N, *et al*. IL-33 reduces the development of atherosclerosis. J Exp Med. 2008; 205:339-46. https://doi.org/10.1084/jem.20071868
41. Miller AM, Asquith DL, Hueber AJ, Anderson LA, Holmes WM, McKenzie AN, *et al*. Interleukin-33 induces protective effects in adipose tissue inflammation during obesity in mice. Circ Res. 2010; 107:650-8. https://doi.org/10.1161/CIRCRESAHA.110.218867
42. Villacorta H, Maisel AS. Soluble ST2 testing: A promising biomarker in the management of heart failure. Arq Bras Cardiol. 2016; 106(2):145-52. https://doi.org/10.5935/abc.20150151
43. Seki K, Sanada S, Kudinova AY, Steinhauser ML, Handa V, Gannon J, *et al*. Interleukin-33 prevents apoptosis and improves survival after experimental myocardial infarction through ST2 signaling. Circ Heart Fail. 2009; 2(6):684-91. https://doi.org/10.1161/CIRCHEARTFAILURE.109.873240
44. Bartunek J, Delrue L, Van Durme F, Muller O, Casselman F, De Wiest RNB, *et al*. Nonmyocardial production of ST2 protein in human hypertrophy and failure is related to diastolic load. J Am Coll Cardiol. 2008; 52:2166-74. https://doi.org/10.1016/j.jacc.2008.09.027

45. Demyanets S, Kaun C, Pentz R, Krychtiuk KA, Rauscher S, Pfaffenberger S, *et al*. Components of the interleukin-33/ST2 system are differentially expressed and regulated in human cardiac cells and in cells of the cardiac vasculature. J Mol Cell Cardiol. 2013; 60:16-26. https://doi.org/10.1016/j.yjmcc.2013.03.020
46. González FEM, Ponce-RuÍz N, Rojas-GarcÍa AE, Bernal-Hernández YY, Mackness M, Ponce-Gallegos J, *et. al*. PON1 concentration and high-density lipoprotein characteristics as cardiovascular biomarkers. Arch Med Sci Atheroscler Dis. 2019; 4: e47-54. https://doi.org/10.5114/amsad.2019.84447
47. Mackness MI, Arrol S, Durrington PN. Paraoxonase prevents accumulation of lipoperoxides in low-density lipoprotein. FEBS Lett. 1991; 286:152-4. https://doi.org/10.1016/0014-5793(91)80962-3
48. Shih DM, Lusis AJ. The roles of PON1 and PON2 in cardiovascular disease and innate immunity. Curr Opin Lipidol. 2009; 20(4):288-92. https://doi.org/10.1097/MOL.0b013e32832ca1ee
49. Grzegorzewska AE, Adamska P, Iwańczyk-Skalska E, Ostromecka K, Niepolski L, Marcinkowski W, *et al.* Paraoxonase 1 concerning dyslipidemia, cardiovascular diseases, and mortality in haemodialysis patients. Sci Rep. 2021; 11. https://doi.org/10.1038/s41598-021-86231-0
50. Shunmoogam N, Naidoo P, Chilton R. Paraoxonase (PON)-1: A brief overview on genetics, structure, polymorphisms and clinical relevance. Vasc Health Risk Manag. 2018; 14:137-43. https://doi.org/10.2147/VHRM.S165173
51. Mansi K, Abushoffa AM, Disi A, Aburjai T. Hypolipidemic effects of seed extract of celery (*Apium graveolens*) in rats. Pharmacogn Mag. 2009; 5:301-5. https://doi.org/10.4103/0973-1296.58149
52. Adegbola P, Aderibigbe I, Hammed W, Omotayo T. Antioxidant and anti-inflammatory medicinal plants have a potential role in the treatment of cardiovascular disease: A review. Am J Cardiovasc Dis. 2017; 7(2):19-32. PMID: 28533927.
53. Dey A, De JN. Pharmacology and medicobotony of aristolochia tagala cham: A review. Pharma Sci Monitor and Int J of Pharm Sci. 2012; 3:0976-7908.
54. Zhi K, Li M, Zhang X, Gao Z, Bai J, Wu Y, *et al*. α4β7 integrin (LPAM-1) is upregulated at atherosclerotic lesions and is involved in atherosclerosis progression. Cell Physiol Biochem. 2014; 33:1876-87. https://doi.org/10.1159/000362965
55. Abotsi WK, Ainooson G, Gyasi EB. Acute and sub-acute toxicity studies of the ethanolic extract of the aerial parts of *Hilleria latifolia* (Lam.) H. Walt. (Phytolaccaceae) in rodents. West Afr J Pharma. 2011; 22:27-35. https://doi.org/10.4314/ajtcam.v9i1.19
56. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without the use of the preparative ultracentrifuge. Clinic Chem. 1972; 18(6):499-502. PMID: 4337382. https://doi.org/10.1093/clinchem/18.6.499
57. Singh M, Pathak MS, Paul A. A study on atherogenic indices of pregnancy induced hypertension patients as compared to normal pregnant women. J Clin Diagn Res. 2015; 9(7):BC05-8. https://doi.org/10.7860/JCDR/2015/13505.6241
58. Sinha AK. Colorimetric assay of catalase. Anal Biochem. 1972; 47:389-94. https://doi.org/10.1016/0003-2697(72)90132-7
59. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys. 1959; 82:70-7. https://doi.org/10.1016/0003-9861(59)90090-6
60. Hwang YP, Choi JH, Jeong HG. Protective effect of the *Aralia continentalis* root extract against carbon tetrachloride-induced hepatotoxicity in mice. Food Chem Toxicol. 2009; 47:75-81. https://doi.org/10.1016/j.fct.2008.10.011
61. Bradford MM. A rapid sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976; 72:248-54. https://doi.org/10.1016/0003-2697(76)90527-3
62. Untergasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M, *et al*. Primer3- New capabilities and interfaces. Nucleic Acids Res. 2012; 40(15). https://doi.org/10.1093/nar/gks596
63. Koressaar T, Remm M. Enhancements and modifications of primer design program Primer3. Bioinformatics. 2007; 23(10):1289-91. https://doi.org/10.1093/bioinformatics/btm091
64. Bonnefoy M, Abidi H, Jauffret M, Garcia I, Surrace J, Drai J. Hypocholesterolemia in hospitalized elderly: Relations with inflammatory and nutritional status. Rev Med Interne. 2002; 23:991-8. https://doi.org/10.1016/S0248-8663(02)00718-X
65. Ainslie DA, Proietto J, Fam BC, Thorburn AW. Short-term, high-fat diets lower circulating leptin concentrations in rats. Am J Clin Nutr. 2000; 71:438-42. https://doi.org/10.1093/ajcn/71.2.438
66. Parry SA, Hodson L. Influence of dietary macronutrients on liver fat accumulation and metabolism. J Investig Med. 2017; 65:1102-15. https://doi.org/10.1136/jim-2017-000524
67. Marbaniang C, Sharan RN, Kma L. *In-vitro* comparative studies of *Apium graveolens* L. extracts

for antioxidant and anti-inflammatory activity. The NEHU J. 2020; 18(1):43-59.
68. Mahnaz K, Cordell GA, Sarker MMR, Radzi CwJBWM, Hajifaraji M, Kiat PE. Alternative treatments for weight loss: Safety/risks and effectiveness of anti-obesity medicinal plants. Int J Food Prop. 2015; 18:1942-63. https://doi.org/10.1080/10942912.2014.933350
69. Sarzani R, Spannella F, Giulietti F, Balietti P, Cocci G, Bordicchia M. Natriuretic peptides, hypertension, and cardiovascular risk. High Blood Press Cardiovasc Prev. 2017; 24:115-26. https://doi.org/10.1007/s40292-017-0196-1
70. Hutcheson R, Rocic P. The vast exploration of the metabolic syndrome, oxidative stress, the environment, and cardiovascular disease. Exp Diabetes Res. 2012. https://doi.org/10.1155/2012/271028
71. Oh, Phil-Sun, Lee S-J, Lim K-T. Hypolipidemic and antioxidative effects of the plant glycoprotein (36 kDa) from *Rhus verniciflua* stokes fruit in triton WR-1339-induced hyperlipidemic mice. Biosci Biotechnol Biochem. 2006; 70:447-56. https://doi.org/10.1271/bbb.70.447
72. Vijayaraj P, Muthukumar K, Sabarirajan J, Nachiappan V. Antihyperlipidemic activity of *Cassia auriculata* flowers in triton WR 1339 induced hyperlipidemic rats. Exp Toxicol Pathol. 2013; 65(1-2):135-41. https://doi.org/10.1016/j.etp.2011.07.001
73. Sugimoto S, Nakamura S, Yamamoto S, Yamashita C, Oda Y, Matsuda H, *et al*. Brazilian natural medicines III. Structures of triterpene oligoglycosides and lipase inhibitors from mate, leaves of Ilex paraguariensis. Chem Pharm Bull. 2009; 57:257-61. https://doi.org/10.1248/cpb.57.257
74. Chander R, Khanna AK, Raj K, Rastogi AK. Antioxidant and lipid-lowering activities of Indian black tea. Indian J Clin Biochem. 2005; 20:153 https://doi.org/10.1007/BF02893063
75. Barter PJ, Nicholls S, Rye K, Anantharamaiah GM, Navab M, Fogelman AM. Antiinflammatory properties of HDL. Circ Res. 2004; 95:764-72 https://doi.org/10.1161/01.RES.0000146094.59640.13
76. Maryam K, Mehdi M, ZohrehR, Yahya P, Ebrahim S. Liver enzymes and their association with some cardiometabolic diseases: Evidence from a large kurdish cohort. Bio Med Res Int. 2021; 2021:1-8. https://doi.org/10.1155/2021/5584452
77. Levitan EB, Song Y, Ford ES, Liu S. Is nondiabetic hyperglycemia a risk factor for cardiovascular disease? A meta-analysis of prospective studies. *Arch Intern Med*. 2004; 164(19):2147-55. https://doi.org/10.1001/archinte.164.19.2147
78. Marshall JA, Bessesen DH. Dietary fat and the development of Type 2 diabetes. *Diabetes Care*. 2002; 25(3):620-2. https://doi.org/10.2337/diacare.25.3.620
79. Hanhineva K, Törrönen R, Bondia-Pons I, Pekkinen J, Kolehmainen M, Mykkänen H, *et al*. Impact of dietary polyphenols on carbohydrate metabolism. Int J Mol Sci. 2010; 11(4):1365-402. https://doi.org/10.3390/ijms11041365
80. Yang JJ, Li GL, Liu HR., Ren BB. Effect of evening rose oil on activities of oxygen-free radical scavenging-related enzymes and hepatic morphosis in rats on high lipid diet. J NingXia Med Coll. 2003; 25:244-6.
81. Ilaria D, Adriana V, Emilia F, Bruno B. Combined effects of high-fat diet and ethanol induce oxidative stress in rat liver. Alcohol. 2006; 40(3):185-191. https://doi.org/10.1016/j.alcohol.2006.12.006
82. Panchal S, Poudyal H, Iyer A, Nazer R, Alam A, Diwan V, *et al*. High-carbohydrate, high-fat diet-induced metabolic syndrome and cardiovascular remodeling in rats. J Cardiovasc Pharmacol. 2011; 57(5):611-24. https://doi.org/10.1097/FJC.0b013e3181feb90a
83. Huang L, Heinloth A, Zeng Z-B, Paules R, Bushel P. Genes related to apoptosis predict necrosis of the liver as a phenotype observed in rats exposed to a compendium of hepatotoxicants. BMC Genomics. 2008; 9(1):288. https://doi.org/10.1186/1471-2164-9-288
84. Fraser A, Harris R, Sattar N, Ebrahim S, Smith GD, Lawlor DA. Gamma-glutamyltransferase is associated with incident vascular events independently of alcohol intake: Analysis of the British women's heart and health study and meta-analysis. Arterioscler Thromb Vasc Biol. 2007; 27:2729-35. https://doi.org/10.1161/ATVBAHA.107.152298
85. McArdle PF, Whitcomb BW, Tanner K, Mitchell BD, Shuldiner AR, Parsa A. Association between bilirubin and cardiovascular disease risk factors: Using Mendelian randomization to assess causal inference. BMC Cardiovasc Disord. 2012; 12:16. https://doi.org/10.1186/1471-2261-12-16
86. Zhu W, Ma Y, Guo W, Lu J, Li X, Wu J, *et al*. Serum level of lactate dehydrogenase is associated with cardiovascular disease risk as determined by the framingham risk score and arterial stiffness in a health-examined population in China. Int J Gen Med. 2022; 15:11-17. https://doi.org/10.2147/IJGM.S337517
87. Rahimi-Sakak F, Maroofi M, Rahmani J, Bellissimo N, Hekmatdoost A. Serum uric acid and risk of cardiovascular mortality: A systematic review and dose-response meta-analysis of cohort studies of over

a million participants. BMC Cardiovasc Disord. 2019; 19:218. https://doi.org/10.1186/s12872-019-1215-z
88. Prasad K. Reduction of serum cholesterol and hypercholesterolemic atherosclerosis in rabbits by secoisolariciresinol diglucoside isolated from flaxseed. Circulation. 1999; 99:1355-62. https://doi.org/10.1161/01.CIR.99.10.1355
89. Rocha KKR, Souza GA, Ebaid GX, Seiva FRF, Cataneo AC, Novelli ELB. Resveratrol toxicity: Effects on risk factors for atherosclerosis and hepatic oxidative stress in standard and high-fat diets. Food Chem. Toxicol. 2009; 47:1362-7. https://doi.org/10.1016/j.fct.2009.03.010
90. Chance B, Sies H, Boveris A. Hydroperoxide metabolism in mammalian organs. Physiol Rev. 1979; 59(3):527-605. https://doi.org/10.1152/physrev.1979.59.3.527
91. Cui BK, Liu S, Lin XJ, Wang J, Li SH, Wang QB, *et al*. Effects of *Lycium barbarum* aqueous and ethanol extracts on high-fat-diet induced oxidative stress in rat liver tissue. Molecules. 2011; 16:9116-28. https://doi.org/10.3390/molecules16119116
92. Anuradha CV, Selvam R. Effect of oral methionine on tissue lipid peroxidation and antioxidants in alloxan-induced diabetic rats. J Nutr Biochem. 1993; 4(4): 212-17. https://doi.org/10.1016/0955-2863(93)90054-Z
93. Ntchapda F, Maguirgue K, Adjia H, Etet PFS, Dimo T. Hypolipidemic, antioxidant and anti-atherosclerogenic effects of the aqueous extract of *Zanthoxylum heitzii* stem bark in diet-induced hypercholesterolemic rats. Asian Pac J Trop Med. 2015; 359-65. https://doi.org/10.1016/S1995-7645(14)60344-8
94. Opoku AR, Maseko NF, Terblanche SE. The *in vitro* antioxidative activity of some traditional Zulu medicinal plants. Phytother Res. 2002; 16:51-6. https://doi.org/10.1002/ptr.804
95. Libby P. Inflammation and cardiovascular disease mechanisms. Am J Clin Nutr. 2006; 83:456S–60S. https://doi.org/10.1093/ajcn/83.2.456S
96. Ridker M, Everett BM, Thuren T, MacFadyen JG, Chang WH, Ballantyne C, *et al*. Antiinflammatory therapy with canakinumab for atherosclerotic disease. N Engl J Med. 2017; 377:1119-31. https://doi.org/10.1056/NEJMoa1707914
97. Sánchez-Más J, Lax A, Model CA-L, *et al*. Correlation between cardiac re-modeling markers and modulation of the IL-33/ST2 system in post-infarction heart failure. EJCI. 2014; 44:643-51. https://doi.org/10.1111/eci.12282
98. Song W, Wang H, Wu Q. Atrial natriuretic peptide in cardiovascular biology and disease (NPPA). Gene. 2015; 569(1):1-6. https://doi.org/10.1016/j.gene.2015.06.029
99. Kerkel€a R, Ulvila J, Magga J. Natriuretic peptides in the regulation of cardiovascular physiology and metabolic events. J Am Heart Assoc. 2015; 4. https://doi.org/10.1161/JAHA.115.002423