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Research Article

Caesalpinia bonduc (L) Roxb Seed Extract Dosage and Sex Effects on Pathobiological Changes in A Rat Model of Diabetes Mellitus

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Abstract
Caesalpinia bonduc (L) Roxb is a medicinal plant commonly utilized in traditional Indonesian medicine, particularly for the treatment of diabetes mellitus (DM). This study examined the effects of varying doses C. *bonduc* extract on fasting blood sugar (FBS), malondialdehyde (MDA), tumor necrosis factor-α (TNF-α), transforming growth factor-β (TGF-β), and pancreatic histology in male and female wistar rats with diabetes. The study design included six groups of 18 male and female rats (Wistar strain) each. To develop diabetes, nicotinamide (NA) (110 mg/kg) and streptozotocin (STZ) (45 mg/kg) were supplied, followed by oral administration of C. *bonduc* extract at dosages of 200, 400, and 600 mg/kg for 21 days. We utilized ELISA to analyze biochemical parameters and employed H&E staining to evaluate histological changes. A factorial design was employed for the statistical analysis to examine the effects of both dosage and sex. This study offers a unique comparative examination of the antidiabetic efficacy of C. *bonduc* across genders, a dimension seldom explored in experimental diabetes research. The findings indicated that C. *bonduc* markedly reduced levels of FBS, MDA, TNF-α, and TGF-β ($p < 0.05$) enhanced the structural integrity of the pancreas. There were no significant differences between males and female ($p > 0.05$). The dosage and sex influenced MDA and TGF-β. The extract of C. *bonduc* shown the ability to lower blood sugar, fight free radicals, and reduce inflammation; however, the intensity of these benefits depended on the dosage given. This means that it might assist persons with diabetes minimize oxidative stress and damage to the pancreas.

Keywords: *Caesalpinia bonduc*; diabetes mellitus; dose; pathobiology; sex

1. INTRODUCTION

Diabetes mellitus (DM) is a complex condition that causes continuous high blood sugar levels because the body doesn't make enough insulin, which causes metabolic problems [1]. According to the International Diabetes Federation (IDF, 2024), an estimated 537 million adults aged 20–79 years were living with diabetes in 2021, and this number has increased to approximately 589 million in 2024. By 2045, the global prevalence is projected to reach 783 million people [2]. Indonesia is ranked fifth in the world for the most diabetes cases. According to the results of the 2023 Indonesian Health Survey (SKI), the prevalence of DM based on blood sugar level checks in the population aged ≥ 15 years is 1.3% for men and 2.0% for women [3]. This metabolic disorder needs continuous medication, leading to several side effects [4]. In 2024, global diabetes-related health expenditure surpassed one trillion US dollars for the first time, up from $966 billion in 2021 [2]. As a result, there is a need for new anti-diabetic options that are cheaper and have fewer side effects, especially those that come from plants [5].

Long-term high blood sugar levels will start different signaling pathways, like the polyol pathway and advanced glycation end products (advanced glycation end products (AGEs)). These changes will make the enzyme nicotinamide adenine dinucleotide phosphate (NADPH) oxidase work harder, which can make reactive oxygen species (ROS) and oxidative stress levels go up, which can cause inflammation [6]. ROS will react with polyunsaturated fatty acids (PUFAs) from the lipid layer of the cell membrane, causing lipid peroxidation. Lipid peroxidation creates malondialdehyde (MDA), a very reactive aldehyde, as well as other toxic aldehydes. It serves as a crucial marker for oxidative stress, according to research [7]. Hurt inhibitor kappa β (ikβ), which can then turn on nuclear factor kappa β (NF-kβ). Elevated proinflammatory cytokines, in conjunction

(**a**) (**b**) (**c**)

Figure 1. (a) Plant, (b) fruit, and (c) seed of *Caesalpinia bonduc* (L) Roxb.

with oxidative stress, may result in pancreatic β-cell injury. Death can happen if harm continues [6][8]. Death of pancreatic β-cells can cause insulin expression to go down, which can then cause insulin secretion to go down even more. A sustained reduction in insulin secretion can exacerbate hyperglycemia, rendering it progressively unmanageable and causing damage to pancreatic β cells [9]. Consequently, conventional medicinal plants possessing antioxidant and anti-inflammatory characteristics are believed to mitigate oxidative stress and lower the levels of fasting blood sugar (FBS), MDA, tumor necrosis factor-α (TNF-α), and transforming growth factor-β (TGF-β) [10]. Oxidative stress is a major cause of β -cell damage and diabetes progression. Therefore, plant-based antioxidants that can change the body's defense systems, such as increasing superoxide dismutase (SOD) and catalase (CAT) activity or stopping lipid peroxidation, are thought to be good medicines.

Ethnomedicine is becoming more popular around the world, and several plants have been utilized to cure and manage diabetes. This is *Caesalpinia bonduc* (L) Roxb, which belongs to the *Fabaceae* family and may be found on the island of Sumatra, Indonesia, notably in the Bengkulu region [11]. sterols. It also contains oils, phenols, glycosides, tannins, and resins [12]. The activity of *C. bonduc* as a total antioxidant (24.96 ± 0.31) and its free radical scavenging activity (170 ± 4.08 µg/ mL) may be attributed to phenolic compounds [13]. The ethanol extract of C. *bonduc* seeds has a total phenolic content of 26.99 µg GAE/mg [14]. The total flavonoid concentration of *C. bonduc* is 16.28 µg CE/mg, and it also has antioxidant properties [15].

Given the increasing global burden of diabetes mellitus, there is a growing interest in identifying plant-derived agents with effective antidiabetic potential. Several medicinal plants, such as *Momordica charantia*, *Gymnema sylvestre*, *Trigonella foenum-graecum*, and *Syzygium cumini*, have been widely investigated for their hypoglycemic and antioxidant effects [16][17]. However, *C. bonduc* was selected in this study due to its distinctive phytochemical profile, rich in flavonoids, alkaloids, saponins, and phenolic compounds that possess high antioxidant capacity and inhibitory effects on oxidative and inflammatory pathways linked to diabetes [18]. Prior studies have demonstrated that *C. bonduc* seed extract not only lowers blood glucose but also improves pancreatic β-cell regeneration and modulates lipid metabolism more effectively than many other herbal agents [19][20]. These attributes make *C. bonduc* a promising candidate for developing plant-based therapeutic alternatives with dual antioxidant and antidiabetic potential.

The *C. bonduc* extract is effective because it can increase insulin production, fix damaged pancreatic β-cells, and regulate key genes in the insulin signaling system, including pancreatic and duodenal homeobox 1 (Pdx-1), Ins-1, and glucose transporter type 4 (GLUT-4) [21][22]. It also helps the body break down carbs by making enzymes like glucose-6-phosphate dehydrogenase and hexokinase work better [23]. Some studies use male animals instead of female ones to avoid hormonal factors that could make the results less reliable [24]. Type 2 diabetes

is somewhat more prevalent in males (6.9%) than in females (5.9%) [25]. There is insufficient specific evidence to demonstrate that diabetes treatment varies between men and women, and researchers do not completely comprehend the impact of sex hormones on diabetes at the cellular level [26]. However, comparing male and female rats is about more than just noticing sexual differences. Estrogen and testosterone are two examples of sex hormones that are known to have an effect on oxidative stress, the production of inflammatory cytokines, and insulin sensitivity. This may result in varied biochemical and histological responses to antidiabetic medications.

Therefore, it is imperative to assess both genders to ascertain whether the antioxidant and antidiabetic attributes of C. *bonduc* are sex-dependent, thereby enhancing the comprehension of its therapeutic potential. The goal of this study was to look into how the extract of *C. bonduc* affects FBS, MDA, TNF-α, TGF-β levels, and pancreatic histology in both male and female diabetic model rats. This research offers a unique contribution by explicitly evaluating the differential responses of men and women to C. *bonduc* as an antidiabetic and antioxidant. This is a gap in experimental diabetes research that isn't looked at very often. The hypothesis posited that C. *bonduc* extract would enhance biochemical and histological parameters in a dose-dependent manner, with potential variations influenced by sex. Because of this, the subject interested scholars, which led to this investigation.

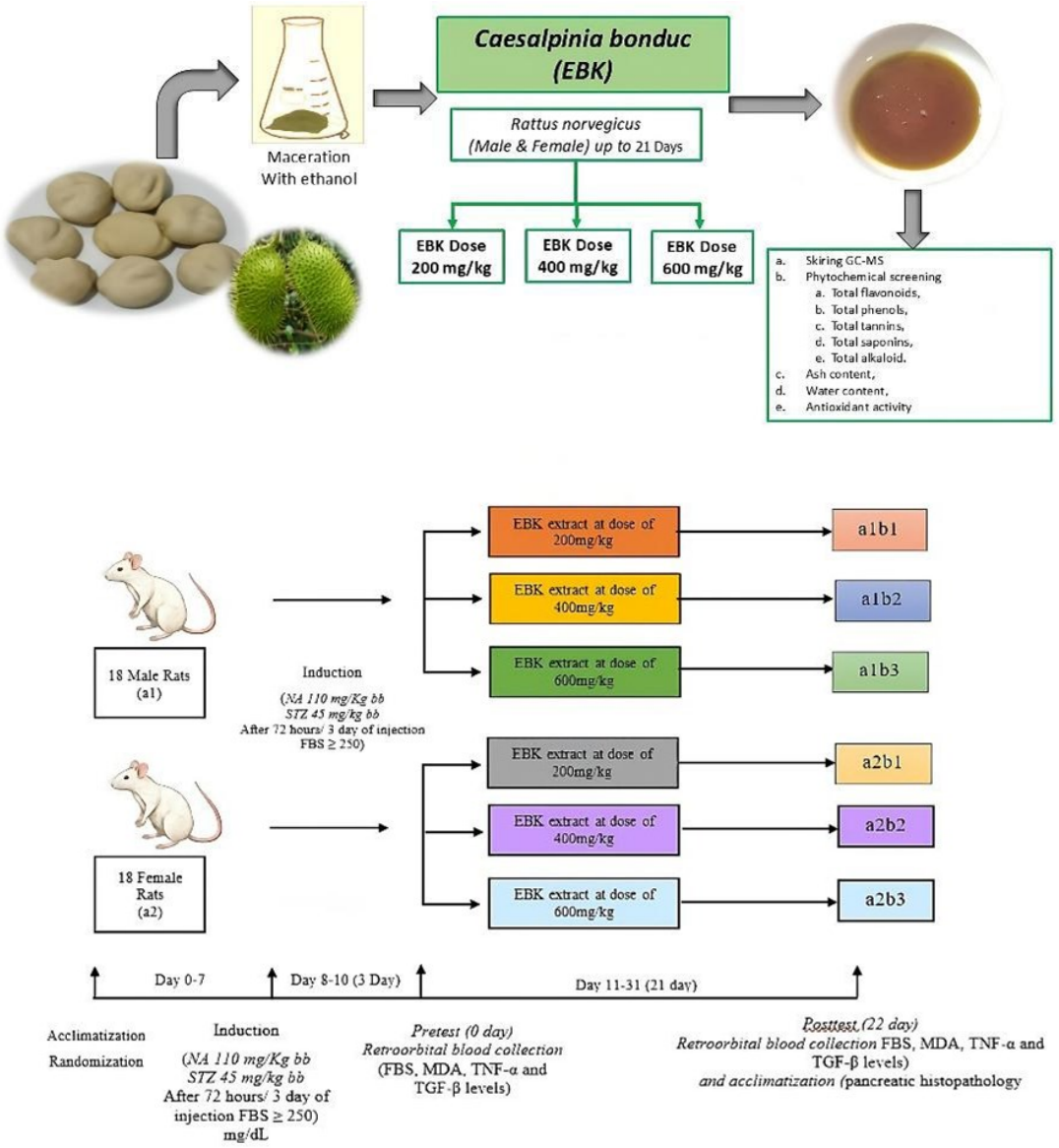


Figure 2. Research experimental design.

Table 1. Phytochemical examination of the ethanol extract from *Caesalpinia bonduc* (L) Roxb seeds.

No	Phytochemical Analysis	Equivalen	Levels (%) mg/g
1	Flavonoid	Quersetin (QE)	1.5997
2	Phenol	Gallic acid (GAE)	14.9103
3	Tannin	Tannic acid (TAE)	7.2144
4	Alkaloid	Quinine	0.0754
5	Saponin	Quillaja bark	3.6105

The goal of this study was to find out how different doses of *C. bonduc*, along with the sex of diabetic mellitus model rats, affected the lowering of FBS, MDA, TNF-α, and TGF-β levels, as well as the improvement of pancreatic histology in the rats.

2. MATERIALS AND METHODS

2.1. Materials

Seeds of *C. bonduc* were gathered from multiple forest sites exhibiting comparable environmental conditions in Bengkulu Province, Indonesia, from January to September 2024 (Figure 1). A determination test was performed at the Functional Service Unit for Traditional Health Services, Tawangmangu, Dr. Sarjito General Hospital, Karanganyar Regency, Central Java, with the report number TL.02.04/D.XI.6/22566.1061/ 2024. Laboratory grade ethanol, quercetin, aluminum chloride ($AlCl_3$), sodium nitrite ($NaNO_2$), sodium hydroxide (NaOH), aquadest, ether, hydrogen chloride (HCl), gallic acid, folin-ciocalteu, sodium carbonate (Na_2CO_3), tannic acid, diethyl ether, quinine, chloroform, buffer phosphate, quillaja bark, sulfuric acid (H_2SO_4), 2-diphenyl-1-picrylhydrazyl (DPPH), nicotinamide (NA), streptozosin (STZ), buffer citrate, glucose kit, the enzymatic glucose oxidase-phenol aminoantypiryn (GOD-PAP) kit, thiobarbituric acid reactive substances (TBARS) assay kit, enzyme-linked immunosorbent assay (ELISA) fine test TNF-α and TGF-β, Weigert's iron hematoxylin-A solution, and Weigert's iron hematoxylin-B solution.

2.2. Methods

2.2.1. Preparation of Ethanol Extract

The seeds of *C. bonduc* were dried powdered, and macerated in ethanol (1:10 w/v) to obtain the crude extract. The solvent was renewed to optimize yield, and the combined filtrates were concentrated under reduced pressure at 50 °C [27]. The seed extract of *C. bonduc* underwent phytochemical screening to identify its components, secondary metabolites, antioxidant activities and chromatography gas–mass spectrometry (GC-MS).

2.2.2. Phytochemical Analysis

2.2.2.1. Qualitative Phytochemical Screening of Secondary Metabolites

The ethanolic extract of *C. bonduc* seed underwent preliminary qualitative phytochemical analysis to identify key groups of secondary metabolite compounds, including alkaloids, phenols, flavonoids, saponins, and tannins.

2.2.2.2. Antioxidant Test with DPPH Method

The sample (1–2 g) was weighed and dissolved in methanol at a specific concentration. A 1 mL aliquot of the mother liquor was transferred to a test tube, to which 1 mL of 200 µM DPPH solution was added. The mixture was incubated in a dark room for 30 min and subsequently diluted to 5 mL with methanol. A blank was prepared using 1 mL of DPPH solution and 4 mL of ethanol. Finally, the absorbance was measured at a wavelength of 515 nm (Equation 1) [28].

$$\text{Total Antioxidant (\%)} = \frac{\text{OD of Blank} - \text{OD of Sample}}{\text{OD of Blank}} x\ 100\% \quad \textbf{(1)}$$

2.2.3. GC-MS Based Profiling

Chemical constituents of the ethanolic extract from C. *bonduc* seeds were analyzed using gas chromatography–mass spectrometry (GC–MS). The profiling was conducted on the same batch of extract used for *in vivo* testing, ensuring chemical consistency across analyses. Compounds were

identified by comparing their mass spectra with those in the NIST library database.

2.2.4. Molecular Docking Analysis

In silico docking studies were performed using standard computational tools, including LigandScout®, ChemDraw®, and AutoDock Tools®. Ligands identified from GC-MS analysis were docked against key protein targets associated with oxidative stress: MDA (PDB ID : 6VJ3) and TNF-α (PDB ID: 7JRA), TGF-β receptor I (PDB ID: 1VJY), and antioxidants: TGF-β (PDB ID: 1TGJ). Docking accuracy was validated through re-docking of native ligands, ensuring root-mean-square deviation (RMSD) values below 2.0 Å. The reliability of docking parameters was verified using binding energy scoring and hydrogen bond interaction mapping in Discovery Studio Visualizer® [29].

2.2.5. Animal and Ethical Approval

This study employs an *in vivo* experimental laboratory design utilizing male and female Wistar strain rats, averaging 150–250 g in body weight and aged 8 weeks, in compliance with Ethical Clearance Guidelines No. 245/UN27.06.11/KEP/EC/2024 issued by the Research Ethics Committee of the Faculty of Medicine, Sebelas Maret University. Animals were acclimatized under standard laboratory conditions with free access to food and water. Randomization was performed using a computer-generated sequence, and outcome assessors were blinded to treatment groups during data collection and histopathological analysis to minimize experimental bias.

2.2.6. Preparation of Diabetic Animals

After 15 min, test animals were given 110 mg/kg of NA and then 45 mg/kg of STZ through the peritoneum. The enzymatic Glucose Oxidase-Phenol Aminoantypiryn (GOD-PAP) test was used to determine the blood glucose level after fasting. If FBS levels were ≥ 250 mg/dL three days after the rats were given STZ and NA, they were said to have DM.

2.2.7. Experimental Design

A two-factor factorial design was employed to evaluate the effects of sex and dosage of *C. bonduc* seed ethanol extract on pancreatic histopathology in diabetic rats. A total of 36 Wistar rats (18 males and 18 females) were randomly assigned into six treatment groups (n = 6 per group). The male rats in group a1b1 were administered 200 mg/kg of the ethanol extract from *C. bonduc* seed. Group a1b2 male rats received a treatment of 400 mg/kg of the ethanol extract from *C. bonduc* seed. Group a1b3 male rats were administered extract ethanol from *C. bonduc* seed at a dosage of 600 mg/kg. Group a2b1 female rats received a 200 mg/kg dosage of extract ethanol from *C. bonduc* seed. Group a2b2 female rats received 400 mg/kg of extract ethanol from *C. bonduc* seed. The group of female rats a2b3 had a dose of 600 mg/kg of extract ethanol from *C. bonduc* seed. All treatment regimens commenced three days post-diabetes induction and persisted daily for 21 days (Figure 2).

2.2.8. Biochemical Measurement

FBG, serum MDA, TNF-α, and TGF-β levels were analyzed using spectrophotometric and ELISA

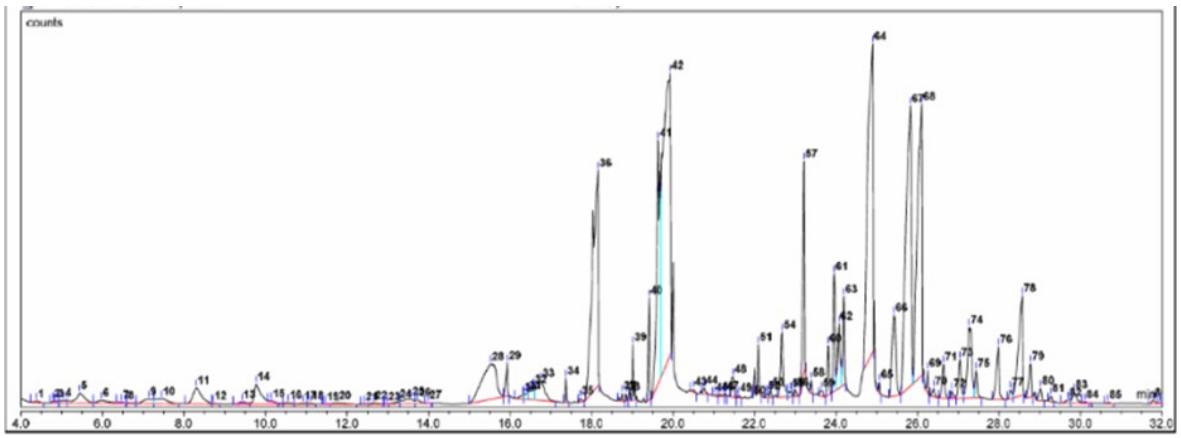


Figure 3. GC-MS chromatogram ethanolic extract of *Caesalpinia bonduc* (L) Roxb seed.

Table 2. Five compounds in the the ethanolic extract of *Caesalpinia bonduc* (L) Roxb seeds with the highest relative peak areas.

No	Name	RT* (Min)	Peak Area (%)	Molekuler Formula	Molecular Weight	SI (NIST)
1	6-Octadecenoic acid	19.93	16.28	$C_{18}H_{34}O_2$	282	877
2	Pregnenolone	24.90	11.77	$C_{21}H_{32}O_2$	316	720
3	1*H*-Cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9a-tetrol, 3-[(acetyloxy)methyl]-1a,1b,4,4a,5,7a,8,9-octahydro-1,1,6,8-tetramethyl-, [1a*R*-(1aa,1bß,4aß,5ß,7aa,7ba,8a,9ß,9aa)]-5,9,9a-triacetate	26.11	9.58	$C_{28}H_{38}O_9$	518	722
4	l-(+)-Ascorbic acid 2,6-dihexadecanoate	18.16	8.97	$C_{38}H_{68}O_8$	652	856
5	Ethyl 9,12-octadecadienoate	19.64	5.86	$C_{20}H_{36}O_2$	308	886

methods according to the manufacturer's protocols.

2.2.9. Histopathological Examination of the Pancreas

After 21 doses of extract ethanol from *C. bonduc* seed, the mice were ready for anesthesia. A dose of 6–100 mg/kg of ketamine was used to euthanize all mice. Afterward, the mice were dissected and their pancreases were removed. Histopathological examination of the mouse pancreas used the hematoxylin and eosin (H&E) staining method. To prepare for pancreatic histopathology, you need to perform a necropsy, isolate the sample, preserve it, dry it, clear it, embed it, section it, stain it, and observe it under a light microscope.

2.2.10. Statistical Analysis

All quantitative data, including FBS, MDA, TNF -α, TGF-β, and pancreatic histopathology scores, were expressed as mean ± standard deviation (SD). A two-way analysis of variance (ANOVA) was performed according to a 2 × 3 factorial design, with sex (male and female) as the first factor and dosage of *C. bonduc* extract (200, 400, and 600 mg/kg) as the second factor. The ANOVA tested both main effects (sex and dosage) and their interaction effect (sex × dosage) on the dependent variables. When significant differences were observed, Tukey's HSD post hoc test was used for pairwise comparisons. All statistical analyses were conducted using SPSS version 25.0 and a value of $p < 0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSIONS

3.1. Phytochemical Analysis

3.1.1. Phytochemical Screening of Secondary Metabolites

The phytochemical screening of the ethanol extract from *C. bonduc* seeds was analyzed using standard methods for the qualitative measurement of secondary metabolite compounds. Table 1 indicates that the ethanol extract from *C. bonduc* seed contains various secondary metabolites, such as flavonoids, phenols, tannins, alkaloids, and saponins.

The ethanol extract of C. *bonduc* seeds has a total phenolic content of 26.99 µg GAE/mg [14]. The total flavonoid concentration of *C. bonduc* is 16.28 µg CE/mg, and it also possesses antioxidant properties [15]. The saponin chemicals in *C. bonduc* can stop blood sugar levels from rising by blocking the α-glucosidase enzyme from working. Saponins stop the glucose transporter system from working, which stops small food molecules like glucose from being absorbed [30].

3.1.2. Antioxidant Activity

The antioxidant capacity of the ethanolic extract of *C. bonduc* seed was evaluated using a DPPH radical scavenging assay and compared with ascorbic acid as a standard antioxidant. The linear regression equation for the ethanolic extract *C. bonduc* seed was $y = - 0.00638 x + 0.71245$, with a correlation coefficient of $R^2 = 0.99801$, suggesting

Table 3. Results of 2D ΔG binding interaction and amino acid bonds.

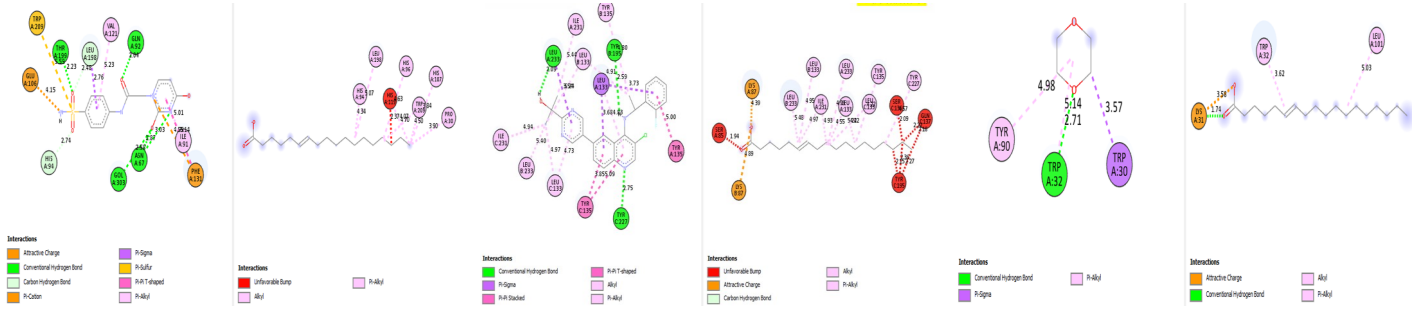
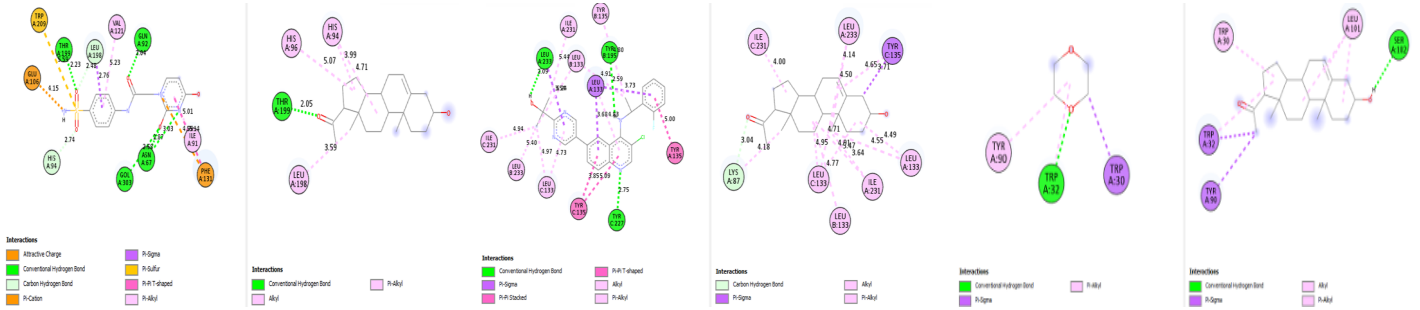
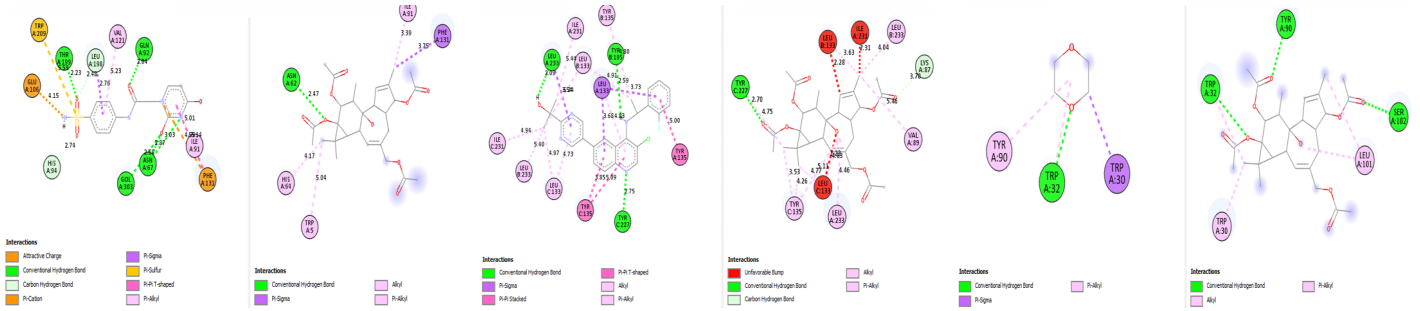
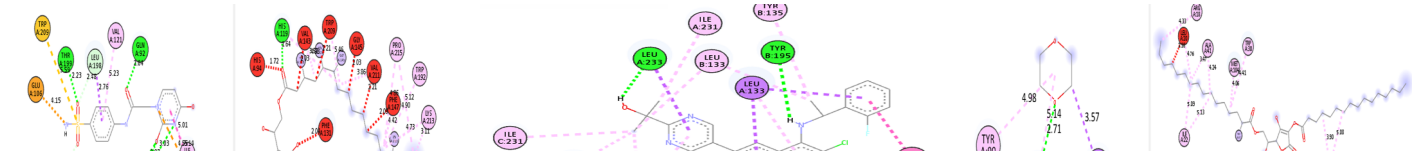
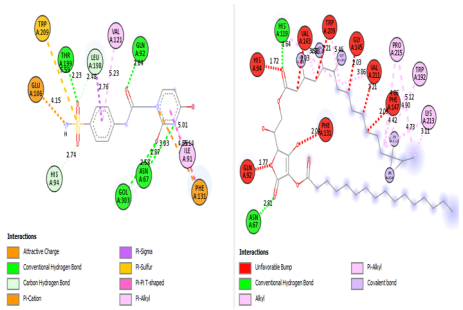
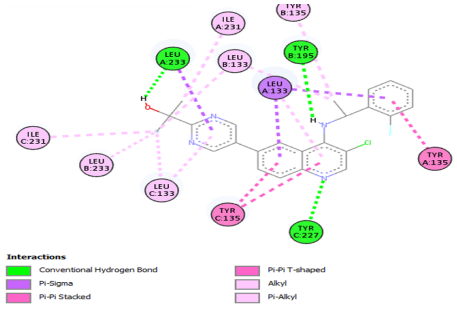
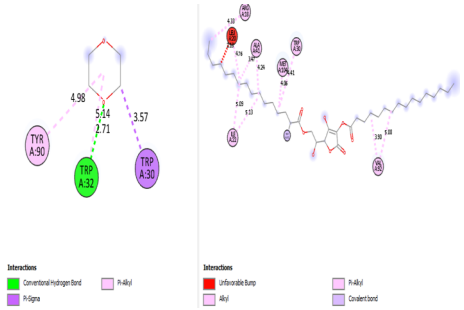
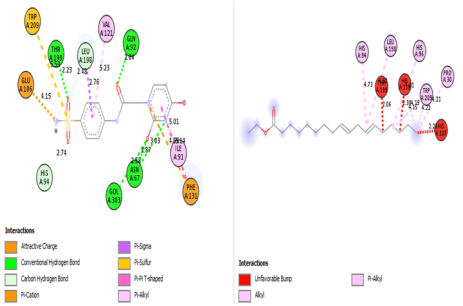
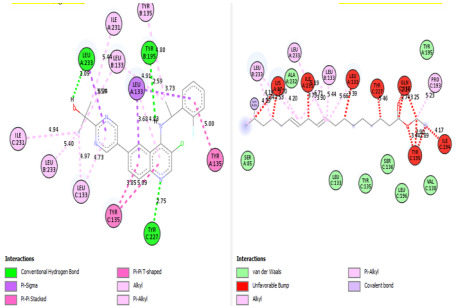
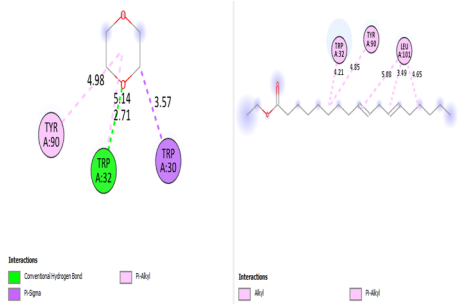
Compound	2D visualization [ΔGBinding interaction(kcal/mol) ± SD]		
	MDA	TNF-α	TGF-β
6-octadecenoic acid			
	13.35± 0.026	66.86 ± 0.025	-0.62 ± 0.000
Pregnenolene			
	-7.54 ± 0.000	-7.98 ± 0.000	-6.20 ± 0.005
(1*R*)-2α,5α,9α,10ßTetraacetoxytaxa-4 (20),11-diene-13-one (cyclopropane)			
	-3.54 ± 5.440	16.52 ± 0.005	-3.77 ± 0.005
l-(+)-Ascorbic acid 2,6 dihexadecanoate			

Table 3. *Cont.*

Compound	2D visualization [ΔGBinding interaction(kcal/mol) ± SD]		
	MDA	TNF-α	TGF-β
l-(+)-Ascorbic acid 2,6 dihexadecanoate			
	5790000 ± 0.000	61866 ± 0.000	6.18 ± 0.000
Ethyl 9,12-Octadecadienoate			
	32.39 ± 0.015	523.85 ± 0.678	12.77 ± 0.040

a strong linear relationship between concentration and antioxidant activity. The antioxidant activity yielded an IC_{50} value of 55.361 ± 0.116 ppm.

These results indicate that ethanolic extract *C. bonduc* seed possesses significant antioxidant potential (IC_{50} ± SD = 55.361 ± 0.116 ppm), which classifies it as a strong antioxidant (< 100 ppm), according to commonly accepted thresholds [31]. This strong antioxidant property may be due to the presence of bioactive compounds like flavonoids, phenols, tannins, saponins, and alkaloids. These compounds are powerful radical scavengers and help improve the histopathology of the pancreas in T2DM rats.

A prior investigation evaluated both a chloroform extract of *C. bonduc* seeds and conventional ascorbic acid for in vitro antioxidant activity using the DPPH method. The chloroform extract exhibited antioxidant activity with an IC_{50} value of 170 ± 4.08 µg/mL. The IC_{50} value for ascorbic acid was 2.03 ± 0.16 µg/mL [13]. This suggests that the ethanolic extract of *C. bonduc* seeds has a higher IC_{50} value, which means it has more antioxidant power than the chloroform extract of *C. bonduc* seeds. Flavonoids and phenolics are part of the polyphenol chemicals that act as antioxidants [32]. This is consistent with the findings about the chemicals present in the ethanolic extract of *C. bonduc* seeds, which exhibit antioxidant properties.

3.2. GC-MS based Profiling

To complement the qualitative secondary metabolite findings, GC-MS analysis was performed to identify individual secondary metabolites present in the extract, providing a detailed chemical profile of the bioactive compounds. The results from GC-MS analysis revealed the presence of 87 compounds in the ethanol extract of *C. bonduc* seed, with the highest compound at a retention time of 19.93 min, namely 6-octadecenoic acid (Figure 3).

Other compounds with the highest concentration are l-(+)-ascorbic acid 2,6-dihexadecanoate ($C_{38}H_{68}O_8$), a carbohydrate ester with antioxidant potential; 9,12-ethyl octadecadienoate ($C_{20}H_{36}O_2$); pregnenolone ($C_{21}H_{32}O_2$), classified as a steroid; and 1,6,8-tetramethyl-1*H*-cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9a-tetrol-3-[(acetyloxy)methyl]-1a,1b,4,4a,5,7a,8,9-octahydro1 [1a*R*-(1aa,1bß,4aß,5ß,7aa,7ba,8a,9ß,9aa)]-5,9,9a-triacetate ($C_{28}H_{38}O_9$), a terpenoid. Table 2 summarizes the five compounds in the the ethanolic extract of *C. bonduc* seeds with the highest relative peak areas in this investigation.

3.3. Analysis in Silico

The GS-MS analysis of the ethanolic extract from *C. bonduc* seeds revealed five target chemicals with the highest similarity index (SI) and the most substantial actual image. The chemicals are l-(+)-ascorbic acid 2,6-dihexadecanoate, 6-octadecenoic acid, ethyl 9,12-octadecadienoate, pregnenolone, and 1,6,8-tetramethyl-1*H*-cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9a-tetrol-3-[(acetyloxy)methyl]-1a,1b,4,4a,5,7a,8,9-octahydro1 [1a*R*-(1aa,1bß,4aß,5ß,7aa,7ba,8a,9ß,9aa)]-5,9,9a-triacetate. The target compound's results were examined *in silico* with the target receptors MDA, TNF-α, and TGF-β. The ΔGbinding values can be

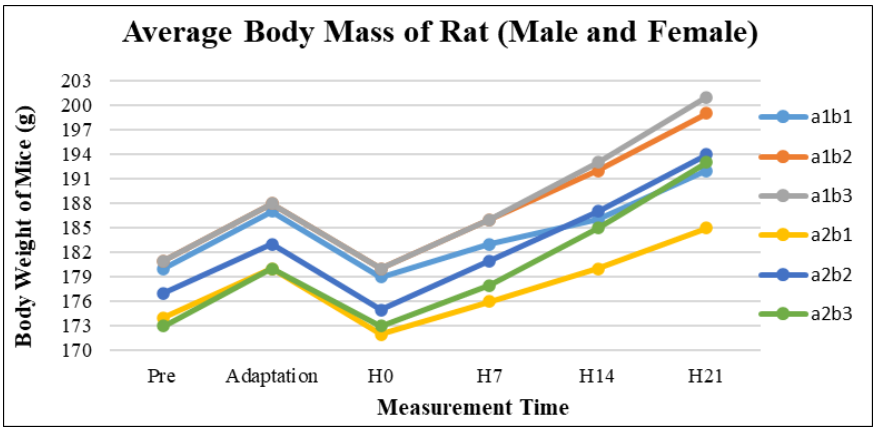


Figure 4. The average body mass of both male and female rats throughout the study.

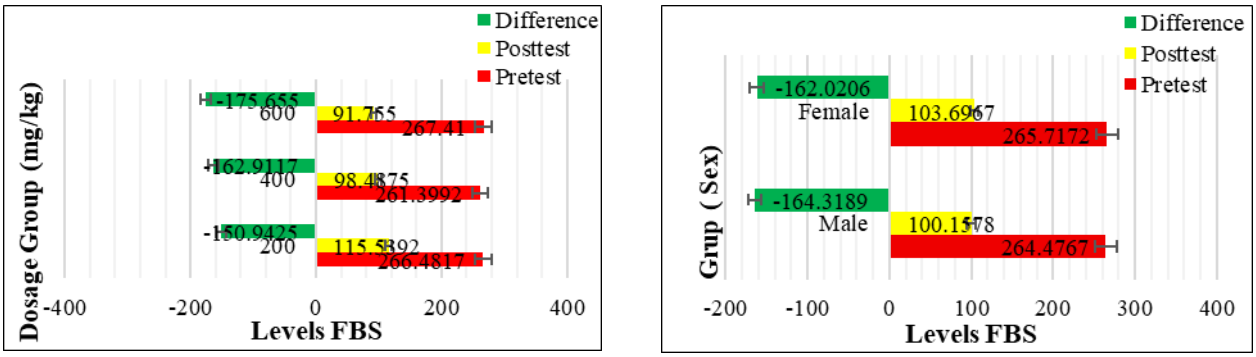


Figure 5. Difference in mean FBS levels pretest and post-test treatment. (a) According to the dose variation of *Caesalpinia bonduc* seed ethanol extract (200, 400, and 600 mg/kg); (b) According to the sex of the rats.

found in Table 3.

The interaction between the target molecule and the receptor is evaluated using ΔG binding, binding constant, and amino acid bonds in relation to the original ligand. If the drug binds to the original ligand in a similar way, has the same amino acid residues as the original ligand, a low ΔG binding energy, and an inhibition constant that shows activity with the original ligand, then it has potential as a drug and can move on to in vitro and in vivo testing. As shown in Table 3, several major phytocompounds identified from *C. bonduc* demonstrated affinity toward MDA, TNF-α, and TGF-β receptor sites. The 6-octadecenoic acid showed affinity energies within the range of -0.62 to 66.86 kcal/mol, with RMSD values of 15.866 Å (MDA), 29.519 Å (TNF-α), and 37.102 Å (TGF-β). Non-hydrogen amino acid interactions ware dominated by residue: Leu198, His94, His96, Trp209, and Pro30 for MDA; Lys87, Leu233, Ile231, Leu133, Tyr135, and Tyr227 for TNF-α, and Trp32, Leu101, and Lys31 for TGF-β.

Pregnenolone exhibited stronger and more consistent affinities ranging from –7.54 to –6.20 kcal/mol, with RMSD values of 17.522 Å (MDA), 28.287 Å (TNF-α), and 37.776 Å (TGF-β). Hydrogen bonding residues included Thr199 (MDA), and Ser102 (TGF-β), while dominant non-hydrogen interactions were Leu198, His94, His96 (MDA), Ile231, Lys87, Leu133, Leu233, Tyr135 (TNF-α), and Trp32, Tyr90, Trp30, Leu101 (TGF-β). The (1*R*)-2α,5α,9α,10β-Tetraacetoxytaxa-4 (20),11-diene-13-one(cyclopropane) also demonstrated good affinity (–3.54 to –16.52 kcal/mol) with RMSD values of 17.414 Å (MDA), 28.752 Å (TNF-α), and 36.706 Å (TGF-β). Key hydrogen bond interactions were found with Asn62 (MDA), Tyr227 (TNF-α), and Trp32, Tyr90, Ser102 (TGF-β). L-(+)-Ascorbic acid 2,6-dihexadecanoate displayed lower stable affinities (6.18 to 5.79×10⁶ kcal/mol), with RMSD values of 19.285 Å (MDA), 29.936 Å (TNF-α), and 34.277 Å (TGF-β). Hydrogen bonds were observed with Asn67, His119 (MDA), and non-hydrogen interactions with Lys213, Pro215, Trp192 (MDA) and Arg18, Ala41, Met104, Trp30, Ile22, Val92 (TGF-β).

Ethyl 9,12-octadecadienoate showed affinity energies between –12.77 and –523.85 kcal/mol, with RMSD values of 16.494 Å (MDA), 29.455 Å (TNF-α), and 35.507 Å (TGF-β). The dominant non-hydrogen interacting residues included His94, Leu198, His96, Trp209, Pro30 (MDA); Leu233, Leu133, Pro193 (TNF-α); and Trp32, Tyr90, Leu101 (TGF-β). However, the RMSD values obtained were higher than the generally accepted docking validation threshold (≤ 2.0 Å), indicating that the binding poses might lack positional stability or that further optimization of grid parameters and ligand flexibility is required.

To enhance interpretability, visualizations of binding sites and ligand–receptor interactions (2D) should be provided in the supplementary material. These should include superimposed poses of docked and native ligands, hydrogen-bond mapping, and interaction surface representations for key receptor targets (MDA, TNF-α, and TGF-β). Overall, among the tested phytocompounds, Pregnenolone and 6-octadecenoic acid demonstrated relatively stronger binding affinities

and consistent amino acid interactions with the receptor binding sites, suggesting their potential as lead antioxidant and anti-inflammatory agents for further in vitro and *in vivo* validation.

3.4. Decrease in Body Mass

Rats that were given STZ-NA exhibited indicators of having diabetes. After three days of induction, the fasting blood sugar levels in all groups went over 250 mg/dL. The scientists also watched the mice's weight. To be included, all of the mice had to weigh between 150 and 250 g. The average weight of the mice during the study showed that each group lost weight when they were given the ethanol extract of *C. bonduc* seed; however, their weight started to go back up after the trial was over.

Figure 4 indicates that all of the T2DM model rats (both male and female) met the inclusion criteria, which is that none of them lost more than 10% of their body mass from their beginning mass. Under H_0, all T2DM model rats (male and female) demonstrated weight loss subsequent to STZ and NA induction, specifically: a1b1 = 8%, a1b2 = 8%, a1b3 = 8%, a2b1 = 9%, a2b2 = 8%, and a2b3 = 8% relative to their initial weight before to STZ-NA induction. This weight loss is one sign that the animal model has developed T2DM. The normality test, which used the Lilliefors or Kolmogorov–Smirnov method, showed that the p-values for the treatment groups receiving ethanolic extract of *C. bonduc* seeds (200, 400, and 600 mg/kg) and for both male and female T2DM rat groups were higher than $\alpha = 0.05$ ($p > 0.05$) for each variable (MDA, TNF-α, and TGF-β). This shows that the data for all treatment groups were distributed in a typical way. We also used Levene's test of homogeneity of variance to check the homogeneity of variances. This test also gave us p-values bigger than $\alpha = 0.05$ ($p > 0.05$). The results show that the pretest, post-test, and difference (Δ) data for each variable (MDA, TNF-α, and TGF-β) had the same variances, which means that the data matched the requirements for two-way ANOVA analysis. Consequently, a two-way ANOVA, succeeded by Tukey's honestly significant difference (HSD) post-hoc test, was used to assess the impacts of dose and sex, together with their interaction, on each biochemical parameter.

3.5. Pathobiological Results

3.5.1. FBS, Oxidative Stress Cytokines, Pro-inflammatory and Antioxidant Factors

The administration of *C. bonduc* seed ethanol extract at varying doses and across different sexes (male and female) will be observed for FBS levels, oxidative stress markers (MDA), pro-inflammatory cytokines (TNF-α), and antioxidants (TGF-β) in all treatment groups.

The study results show that after taking the ethanolic extract of *C. bonduc* seeds, the fasting blood sugar levels went down compared to what they were before treatment. The average drop in glucose levels at a dose of 600 mg/kg (-175.6550 mg/dL) was bigger than the drop in FBS levels at a dose of 400 mg/kg (-162.9117 mg/dL) and the drop in FBS levels at a dose of 200 mg/kg (-150.9425 mg/dL). The ethanolic extract of *C. bonduc* seeds effectively diminished FBS levels in both male and female diabetic rats ($p < 0.05$), contingent upon dose variation. We assessed the external therapeutic efficiency of the ethanolic extract as an antidiabetic drug in reducing fasting blood glucose levels in male and female rats, considering the differences in doses supplied prior to and during therapy (Figure 5).

STZ injected into the peritoneum causes a number of harmful effects on cells, including aberrant deoxyribonucleic acid (DNA) alkylation, protein methylation, and the generation of ROS, reactive nitrogen species (RNS), and ATP depletion. The most common medicine used to make animal models of diabetes mellitus is STZ because diabetes caused by it looks and works like human diabetes. In this investigation, the combination of STZ induction and NA protected the pancreas from significant damage [33]. Nicotinamide is an antioxidant that protects tissues from oxidative damage produced by STZ, preventing extensive tissue damage [34]. Multiple studies have indicated that oxidative stress significantly contributes, both directly and indirectly, to inflammation and tissue damage in the pancreas. Pancreatic islets have heightened vulnerability to oxidative stress compared to other tissues, attributable to their diminished production of antioxidant enzymes. All of these effects of STZ in the mitochondria speed up oxidative stress in

pancreatic β-cells. When free radicals mix with proteins, lipids, and other biological substances, they can start chain reactions that can kill cells [35]. Oxidative stress interacts with inflammation. When immune cells called macrophages are activated in the injured pancreas, they release pro-inflammatory cytokines such as TNF-α, which make pancreatic β-cells less functional and less able to survive [36]. Pro-inflammatory cytokines trigger pancreatic β-cell apoptosis by activating transcription factors, specifically NF-κβ, and by stimulating mitochondrial ROS production and pro-apoptotic components of the intrinsic apoptosis pathway [37] [38]. The activation of NF-κβ can also cause pancreatic β-cells to make more TGF-β [39].

3.5.2. Decrease in Malondialdehyde Levels

The mean alteration in MDA levels pre- and post -treatment at a dosage of 600 mg/kg is (-7.1892 ± 0.6338 nmol/mL). The MDA levels for 400 mg/kg (-5.1775 ± 0.9411 nmol/mL) and 200 mg/kg (-3.3042 ± 0.6752 nmol/mL) are less than this. There was a considerable change in MDA levels before and after testing with different doses of *C. bonduc* (200, 400, and 600 mg/kg). The post hoc test table showed that all dosage groups of *C. bonduc* (200, 400, and 600 mg/kg) for MDA levels had a p-value of less than 0.001, which is less than the significance level of $p < 0.05$ (Tables 4 – 5). The effect was not significant in male and female rats, as evidenced by a p-value of 0.148, which exceeds the threshold of $\alpha = 0.05$ ($p > 0.05$) (Figure 6).

Table 5 and Figure 6(b) shows that the difference in sex between the T2DM model mice did not have a big effect on the decrease in oxidative stress indicators (MDA) in all treatment groups. The p-

Table 4. The Effect of ethanol extract dose administration of *Caesalpinia bonduc* seeds on MDA levels

Dose (mg/kg)	*n*	Difference in MDA Levels		
		Mean	**SD**	***p-value***
200	12	-3.3042	0.6752	<0.001
400	12	-5.1775	0.9411	
600	12	-7.1892	0.6338	

Table 5. The influence of rat`s sex (male and female) on MDA levels.

Grup (Sex)	*n*	Difference in MDA Levels		
		Mean	**SD**	***p-value***
Male	18	-5.6533	1.8863	0.148
Female	18	-4.7939	1.5836	

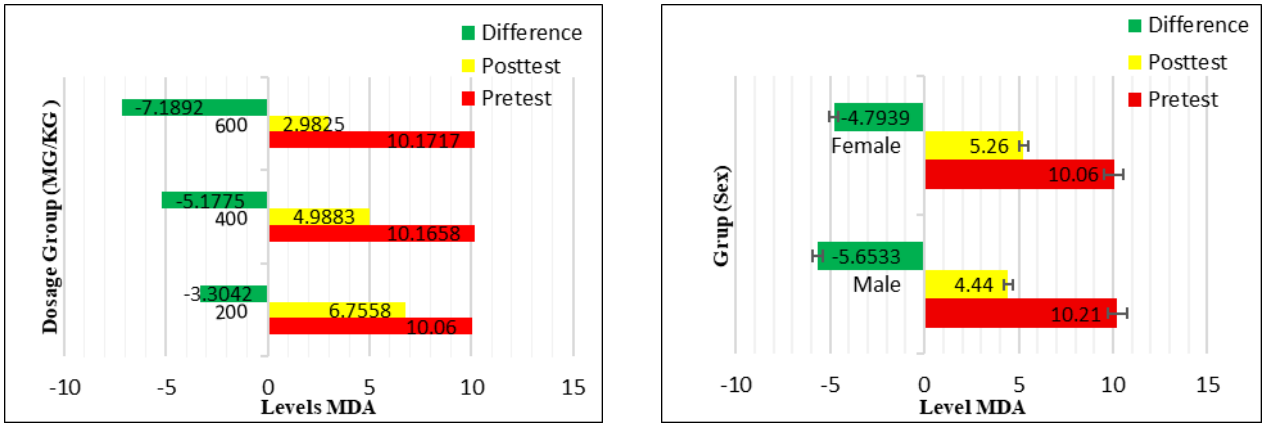


Figure 6. Difference in mean MDA levels pretest and posttest treatment. (a) According to the dose variation of *Caesalpinia bonduc* seed ethanol extract (200, 400, and 600 mg/kg); (b) According to the sex of the rats.

Table 6. Average change in MDA levels.

Sex (a)	Doses (b) (mg/kg) Mean ± SD			p-value
	200 (b1)	400 (b2)	600 (b3)	
Male (a1)	-3.3300±0.6450	-5.9483±0.2635	-7.6817±0.3072	<0.001
Female (a2)	-3.2783±0.7650	-4.4067±0.6731	-6.6967±0.4553	

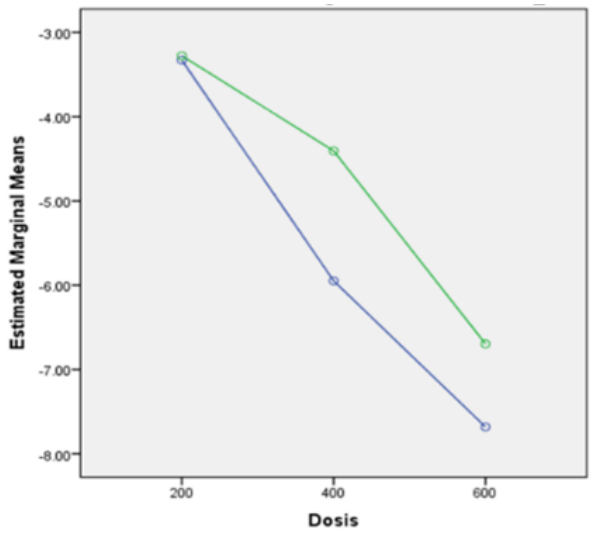


Figure 7. Plot diagram of interaction between doses of *Caesalpinia bonduc* (L) Roxb (200, 400 and 600 mg/kg) and sex of rats (male and female) on the difference of MDA variables.

value was $p > 0.05$. The average reduction in MDA levels in male rats (-5.6533 nmol/mL) was marginally less than that observed in female rats (-4.7939 nmol/mL). The test findings indicated that the disparity in the reduction of MDA levels between male and female rats with T2DM yielded a p-value exceeding $\alpha = 0.05$ ($p > 0.05$). These data indicate that rat sex (male and female) does not influence the reduction of MDA variable levels in T2DM.

Interaction between doses of *C. bonduc* (200, 400 and 600 mg/kg and sex of rats (male and female) on the difference of MDA levels are decreased, with a p-value of less than ($p < 0.05$). Consequently, H1 is accepted while Ho is refused. With a 95% confidence level, these findings indicate that the T2DM model's reduction of MDA levels is influenced by the rat sex (male and female) and the doses of *C. bonduc* (200, 400, and 600 mg/kg). The variations in MDA levels between the six group combinations are regarded as significant (Table 6 and Figure 7).

This phenomenon explains why some rat genders are more affected by the size of the dose given. The diagram above shows an ordinal interaction where the non-parallel lines slope in the same direction but do not actually intersect. This explains that the size of the dose administered has a more intense effect on certain rat genders. A persistent elevation in blood glucose levels will initiate a sequence of actions, resulting in a detrimental cycle. High blood sugar levels and oxidative stress (MDA) will cause inflammation. Subsequently, inflammation will produce additional oxidative stress, exacerbating the existing inflammation. This process will create a vicious cycle between oxidative stress and inflammation [40]. The findings of this study corroborate the research conducted by Zhu et al. [41] and Parawansah et al. [42], which indicated that the administration of STZ and NA in white rats can elicit oxidative stress (ROS). The rise in ROS subsequently induces an elevation in lipid peroxidation, culminating in a substantial increase in blood MDA levels [43]. A greater MDA level means that the test animal is under more oxidative stress [44]. The reduction in FBS, MDA, TNF-α, and TGF-β levels observed in this study may be attributed to the phytochemical constituents,

including total flavonoids 1,599.70 mg/kg eq. quercetin, total phenols 14,910.27 mg/kg eq. gallic acid, alkaloids 75.41 mg/kg eq. quinine, saponins 3,610.47 mg/kg eq. quilaja bark, and tannins 7,214.39 mg/kg eq. tannic acid, derived from *C. bonduc* extract, which have the capacity to inhibit the ROS generation. The antioxidant activity present in *C. bonduc* extract was 91.37%, exhibits therapeutic potential in the DM management by lowering MDA levels, thereby leading to a reduction in glycosylated hemoglobin (FBS) levels. Antioxidants can lower lipid peroxidation caused by Fe^{2+}, which can be a useful treatment for oxidative stress that comes with diabetes [45].

Research conducted by Armenia et al. demonstrated that administration of pure gambir (*Uncaria gambir* Roxb) at various dosages (2.5, 5, and 10 mg/kg) for 14 days in diabetic test rats effectively reduced blood MDA levels [46]. Similar studies demonstrated a reduction in MDA levels in male white wistar rats with T2DM, induced by STZ and NA, and treated with EKORMIN, a combination of okra seeds (*Abelmoschus esculentus*) and turmeric (*Curcuma longa*), administered in three dosage variations: low dose (30.5 mg/kg), medium dose (261 mg/kg), and high dose (522 mg/kg) [47]. The same research was performed on male and female Wistar and Goto-Kakizaki (GK) rats with diabetes models that had ovariectomy as an alternative to estrogen. The rise in oxidative stress was influenced by estrogen levels. The activity was comparable between nondiabetic and diabetic males, although elevated in diabetic females relative to nondiabetic females. However, the activity was greater in males than in females, regardless of diabetes status [48].

Interestingly, in the present study, male rats exhibited a more pronounced reduction in MDA levels at higher doses of *C. bonduc* extract compared to female rats. This difference may be attributed to sex-dependent variations in oxidative metabolism and hormonal regulation. Male rats generally possess higher basal metabolic rates and testosterone-driven ROS production, leading to a greater oxidative burden under diabetic conditions. Consequently, a higher antioxidant dose may be required to effectively neutralize free radicals in males. In contrast, estrogen in female rats provides intrinsic antioxidant protection by upregulating the expression of SOD and catalase, thereby reducing the need for high-dose antioxidant supplementation [49,50]. These findings suggest that the more intense response of male rats at higher doses reflects a compensatory mechanism to counterbalance greater oxidative stress exposure.

3.5.3. Decrease in TNF-α Levels

The TNF-α level at a dose of 600 mg/kg (-17.6208 ± 0,61416 pg/mL) is lower than the TNF-α levels at a dose of 400 mg/kg (-15.2950 ± 0,43548 pg/mL) and a dose of 200 mg/kg (-8.9933 ± 0,40123 pg/mL). The post hoc test table showed that all dosage groups *of C. bonduc* (200, 400, and 600 mg/kg) for TNF-α levels had a p-value of less than 0.001, which is less than the significance level of $p<0.05$ (Tables 7 – 8). The effect was not significant in male and female rats, as evidenced by a p-value of 0.438, which exceeds the threshold of $\alpha = 0.05$ ($p > 0.05$) (Figure 8).

Table 8 and Figure 8(b) shows that the difference in sex between the T2DM model mice did not have a big effect on the decrease in oxidative stress indicators (TNF-α) in all treatment groups. The p-value was $p > 0.05$. The average reduction in TNF-α levels in male rats (-14.2094 pg/mL) were somewhat lower than those in female T2DM rats (-13.7300 pg/mL). The test findings indicated that the disparity in the reduction of TNF-α levels between male and female rats with T2DM yielded a p-value exceeding $\alpha = 0.05$ ($p > 0.05$). These data indicate that rat sex (male and female) does not influence the reduction of TNF-α variable levels in T2DM.

Interaction between doses of *C. bonduc* (200, 400 and 600 mg/kg and sex of rats (male and female) on the difference of TNF-α levels. TNF-α's p-value is equivalent to $\alpha = 0.046$ ($p = 0.05$). Consequently, H_1 is rejected and Ho is accepted. With a 95% confidence level, these findings suggest that there is no interaction between the sex of T2DM rat models and the dose of *C. bonduc* (200, 400, and 600 mg/kg) in terms of lowering TNF-α levels. The image below (Table 9 and Figure 9) shows the interaction plot diagram between the sex of T2DM rat models for TNF-α levels and the dose of *C. bonduc* (200, 400, and 600 mg/kg).

The diagram plot above is useful for assessing whether there is an interaction effect between variables. However, it cannot be used as a valid

reference. It only provides a general overview. When the lines intersect or are not parallel, it indicates interaction. From the diagram above showing parallel lines, it is suspected that there is no interaction effect. The stimulation of STZ will elevate the expression of inflammatory cytokines (TNF-α, IFN-γ, IL-1β) and result in elevated blood glucose levels. The production of TNF-α serves as a critical marker in the acute inflammatory response and is predominantly synthesized by activated mononuclear phagocytes. The inflammatory process in the cell will activate macrophages, which will then make pro-inflammatory cytokines including TNF-α [51].

Bakteryari et al. conducted analogous research on the impact of pioglitazone in lowering TNF-α levels in the serum of female T2DM model rats, demonstrating significantly greater efficacy than metformin [52]. This effect is mediated through the inhibition of TNF-α production from macrophages, suppression of TNF-α mRNA expression in subcutaneous adipose tissue, and a reduction in the population of CD3+ T lymphocytes in diabetic rats. The researchers administered *Ruellia tuberosa* L. *hydroethanol* root extract to diabetic Wistar rats induced by streptozotocin at dosages of 250, 375, and 500 mg/kg for 21 days. The best dose was 250 mg/kg, which lowered TNF-α expression [53]. The extract's potential to lower inflammation is due to direct or indirect effects that lower oxidative stress and hyperglycemia. Flavonoid components in R. *tuberosa* L root extract function as anti-inflammatory agents by inhibiting the expression of proinflammatory cytokines and reducing ROS generation. Flavonoids can stop several proinflammatory cytokines, such as IL-1β, TNF-α,

Table 7. The effect of ethanol extract dose administration of *Caesalpinia bonduc* Seeds on TNF-α levels.

Dose (mg/kg)	*n*	Difference in MDA Levels		
		Mean	SD	*p-value*
200	12	-8.9933	0.40123	
400	12	-15.2950	0.43548	<0.001
600	12	-17.6208	0.61416	

Table 8. The Influence of Rat`s Sex on TNF-α Levels.

Grup (Sex)	*n*	Difference in MDA Levels		
		Mean	SD	*p-value*
Male	18	-14.2094	3.92643	0.438
Female	18	-13.7300	3.61425	

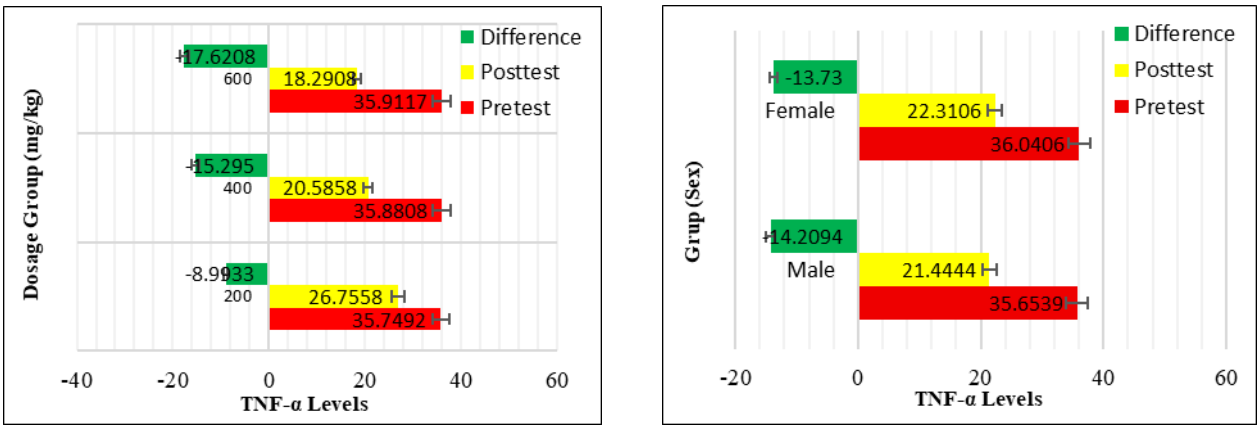


Figure 8. Difference in mean TNF-α levels pre0test and post-test treatment. (a) According to the dose variation of *Caesalpinia bonduc* seed ethanol extract (200, 400, and 600 mg/kg); (b) According to the sex of the rats.

Table 9. Average change in TNF-α levels.

Sex (a)	Doses (b) (mg/kg) Mean ± SD			p-value
	200 (b1)	400 (b2)	600 (b3)	
Male (a1)	-9.0333±0.1415	-15.5083±0.3759	-18.0867±0.2033	0.046
Female (a2)	-8.9533±0.5747	-15.0817±0.4082	-17.1550±0.5174	

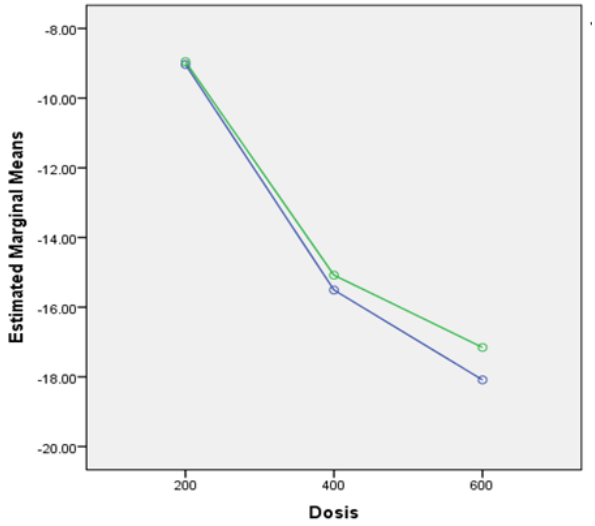


Figure 9. Plot diagram of interaction between doses of *Caesalpinia bonduc* (L) Roxb (200, 400 and 600 mg/kg) and sex of rats (male and female) on the difference of TNF α variables.

and IL-6 [39]. Flavonoids also stop NF-kβ from being active when there are a lot of ROS. A decrease in NF-Kβ activity results in a reduction of TNF alpha expression [54].

In the present study, male rats tended to exhibit a stronger reduction in TNF-α levels at higher doses of *C. bonduc* extract compared to females. This difference may be attributed to sex-related variations in immune and hormonal regulation. Testosterone has been reported to enhance pro-inflammatory cytokine expression, including TNF-α, whereas estrogen exerts anti-inflammatory effects by downregulating NF-κB signaling and reducing macrophage activation [55][56]. Therefore, the higher baseline inflammatory response in males could make them more responsive to the anti-inflammatory effects of the extract at higher doses. Additionally, the flavonoids and alkaloids present in the extract might act synergistically with endogenous estrogen in females, leading to a ceiling effect at moderate doses. This sex-dependent response supports the hypothesis that hormonal regulation plays a key role in modulating TNF-α suppression in diabetic conditions.

3.5.4. Decrease in TGF-β Levels

The TGF-β level at a dose of 600 mg/kg is -7.9800 ± 0,75478 pg/mL, which is greater than the levels at a dose of 400 mg/kg (-6.6450 ± 0,55254 pg/mL) and a dose of 200 mg/kg (-3.7392 ± 0,51407 pg/mL). There was a considerable change in TGF-β levels before and after testing with different doses of *C. bonduc* (200, 400, and 600 mg/kg). The post hoc test table showed that all dosage groups of *C. bonduc* (200, 400, and 600 mg/kg) for TGF-β levels had a p-value of less than 0.001, which is less than the significance level of $p < 0.05$ (Tables 10 – 11). The effect was not significant in male and female rats, as evidenced by a p-value of 0.601, which exceeds the threshold of $\alpha = 0.05$ ($p > 0.05$) (Figure 10).

Table 11 and Figure 10(b) show that the difference in sex between the T2DM model mice did not have a big effect on the decrease in antioxidants (TGF-β) in all treatment groups. The p -value was $p > 0.05$. The average reduction in TGF-β levels in male rats (-6.3317 pg/mL), which was

somewhat higher than the TGF-β levels in female T2DM rats (-5.9111 pg/mL). The test findings indicated that the disparity in the reduction of TGF-β levels between male and female rats with T2DM yielded a p-value exceeding $\alpha = 0.05$ ($p > 0.05$). These data indicate that rat sex (male and female) does not influence the reduction of TGF-β variable levels in T2DM.

Interaction between doses of *C. bonduc* (200, 400 and 600 mg/Kg) and sex of rats (male and female) on the difference of TGF-β levels are decreased, with a p-value of less than ($p < 0.05$). Consequently, H1 is accepted while Ho is refused. With a 95% confidence level, these findings indicate that the T2DM model's reduction of TGF-β levels is influenced by the rat sex (male and female) and the doses of *C. bonduc* (L) Roxb (200, 400, and 600 mg/kg). The variations in TGF-β levels between the 6 group combinations are regarded as significant (Table 12 and Figure 11).

This phenomenon explains why some rat genders are more affected by the size of the dose given. The diagram above shows an ordinal interaction where the non-parallel lines slope in the same direction but do not actually intersect. This explains that the size of the dose administered has a more intense effect on certain rat genders. DM can cause high blood sugar levels, which can make oxidative stress worse. Oxidative stress will hurt Ikβ, which can then turn on NF-kβ. When NF-kβ is turned on, TGF -β levels go up. TGF-β is a multifunctional cytokine that is crucial in numerous cellular activities, such as the regulation of the immune system, growth, differentiation, and the creation of the extracellular matrix. In diabetes, TGF-β has a pathogenic function by facilitating glomerulosclerosis, interstitial fibrosis, and a reduction in glomerular filtration rate (GFR), as well as by elevating the urine excretion of albumin, water, electrolytes, and glucose [57]. An elevation or reduction in TGF-β levels signifies a response aimed at enhancing the body's condition. Studies involving rats with type 1 diabetes indicate that lowering TGF-β levels can mitigate beta cell injury and enhance insulin efficacy [58]. Moreover, the significant elevation in TGF-β and TβR expression during acute pancreatitis indicates a potential role for TGF-β signaling in pancreatic healing. Higher levels of TGF-β may assist in keeping the immune system from becoming active, and they may also support the extracellular matrix, which includes collagen, and the repair of the pancreatic parenchyma [59] [60].

A comparable study by Ritu et al., utilizing a combination therapy of bioflavonoids (ginger extract, soybean extract, and hesperetin) administered orally for 24 weeks to diabetic model rats, indicated that ELISA measurement of TGF-β levels revealed elevated values in diabetic rats compared to normal rats [61]. The bioflavonoid combo treatment, on the other hand, had a big effect on lowering TGF-β in the treated group compared to the diabetic group. The significant amount of antioxidants in the bioflavonoid combo therapy is what makes this happen. Another study utilized pancreatic tissue samples from 6 female and 8 male individuals, with an average age of 65 years (ranging from 37 to 77 years), who underwent NP surgery owing to the onset of acute pancreatitis. There was a significant increase in the expression of TGF-β and its signaling receptors Tβ-RI (ALK5) and Tβ-RII, indicating a potential role for TGF-β in the reparative process following the onset of necrotizing pancreatitis in humans and suggesting that TGF-β may be implicated in the tissue re-modeling and fibrotic responses that occur in the pancreas post-necrosis [62]. Asadikaram et al. conducted a study on normal, opium-addicted, and diabetic male and female rats, revealing that the average serum level of TGF-β in addicted female rats was significantly elevated compared to the control group ($p < 0.004$) [63]. Conversely, in addicted male rats, the average serum level of TGF-β was significantly lower than that of the control group ($p < 0.065$).

Administration of isopsoralen, the active component of Fructus *Psoraleae*, to male and female Wistar rats over a three-month period at doses of 0, 3, 5, 7, 10, and 14 mg/kg resulted in histopathological alterations in pancreatic tissue, accompanied by significant metabolomic changes in both male and female rats, particularly in amino acid metabolism, including the biosynthesis of phenylalanine, tyrosine, and tryptophan, as well as the metabolism of glycine, serine, and threonine. Furthermore, the study primarily influenced fatty acid metabolism in female rats, while lipid metabolism and energy metabolism primarily

affected male rats [64]. Similar to the study by Safitri et al., histological alterations are present in the organs of diabetic rats [65]. The administration of the aqueous root extract of R. *tuberosa* L. at a dosage of 250 mg/kg failed to revert the pathological alterations of the pancreas to a normative state, but a dosage of 500 mg/kg exhibited a mild restoration to a healthy condition. The width of the islets of Langerhans was considerably greater in rats treated at 500 mg/kg compared to diabetic rats.

The study demonstrated that diabetic rats of both genders exhibited similar responses to ethanol extract of *C. bonduc* seeds therapy regarding MDA, TNF-α, and TGF-β levels. Diabetes concurrently affects hormones and metabolism, perhaps leading to indistinguishable effects between genders. Individuals with T2DM may experience dysfunction in their androgen and estrogen pathways due to prolonged elevated blood sugar levels and extended oxidative stress. Both men and women may have metabolic difficulties as a result of this [66][67]. Estrogen is recognized for its ability to counteract free radicals and inhibit the synthesis of cytokines that induce inflammation. But individuals with diabetes couldn't get as many of these benefits because they are under oxidative stress for longer [68]. Men who have high blood sugar levels for a long time may have lower testosterone levels, which could make oxidative damage worse. This would make the redox and inflammatory profiles of both men and women the same [69].

The results show that stress caused by diabetes messes up the hormonal control of oxidative and inflammatory responses, which has consequences

Table 10. The effect of ethanol extract dose administration of *Caesalpinia bonduc* seeds on TGF-β levels.

Dose (mg/kg)	*n*	Difference in MDA Levels		
		Mean	**SD**	***p-value***
200	12	-3.7392	0.51407	
400	12	-6.6450	0.55254	< 0.001
600	12	-7.9800	0.75478	

Table 11. The influence of rat`s sex (male and female) on TGF-β levels.

Grup (Sex)	*n*	Difference in MDA Levels		
		Mean	**SD**	***p-value***
Male	18	-6.3317	2.08123	0.601
Female	18	-5.9111	1.71751	

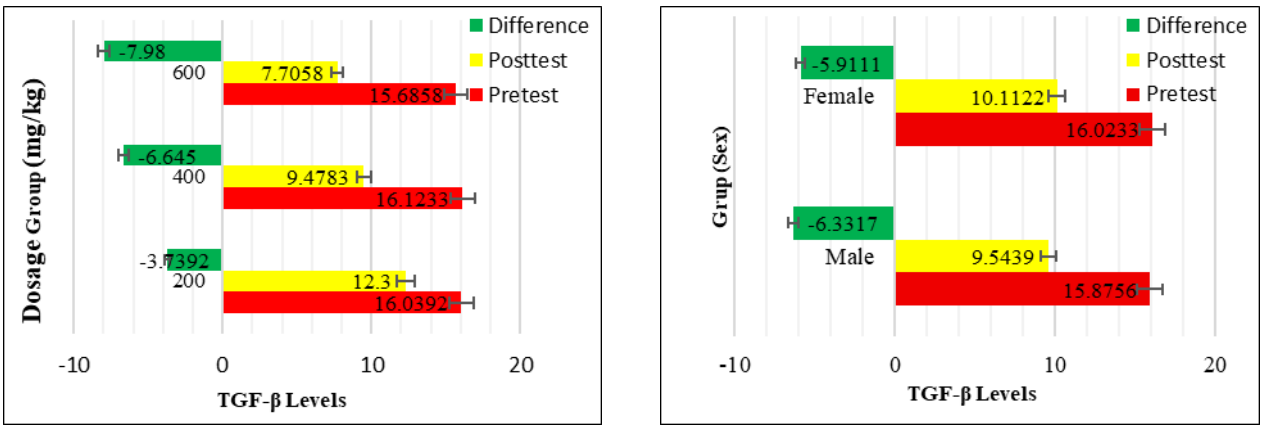


Figure 10. Difference in mean TGF-β levels pre-test and post-test treatment. (a) According to the dose variation of *Caesalpinia bonduc* seed ethanol extract (200, 400, and 600 mg/kg); (b) According to the sex of the rats.

Table 9. Average change in TNF-α levels.

Sex (a)	Doses (b) (mg/kg) Mean ± SD			p-value
	200 (b1)	400 (b2)	600 (b3)	
Male (a1)	-3.6867±0.1727	-6.7250±0.1234	-8.5833±0.1311	<0.001
Female (a2)	-3.7917±0.7382	-6.5650±0.8007	-7.3767±0.6021	

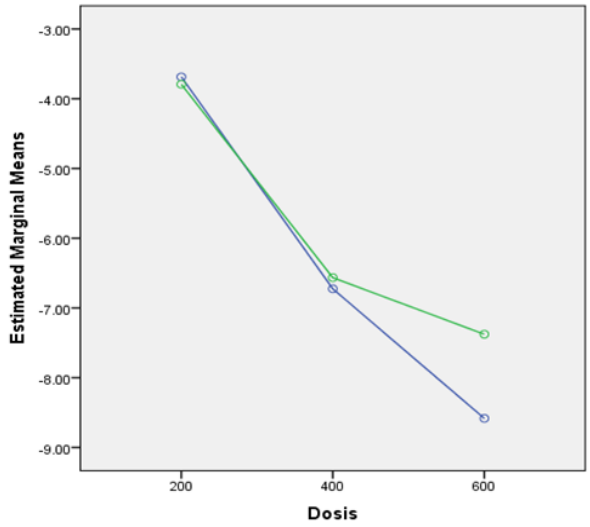


Figure 11. Plot diagram of interaction between doses of *Caesalpinia bonduc* (L) Roxb (200, 400 and 600 mg/kg) and sex of rats (male and female) on the difference of TGF-β variables .

that are similar to those of antioxidant therapy. Thus, the protective effects ethanol extract of *C. bonduc* seeds appear to operate via mechanisms distinct from sex hormones, specifically by altering oxidative and inflammatory pathways common to both sexes [70]. In this study, although both sexes showed a comparable trend in TGF-β reduction, male rats tended to respond more prominently to higher doses of *C. bonduc* extract. This may be due to sex-specific regulation of TGF-β signaling pathways. Previous research indicates that estrogen can attenuate TGF-β/Smad activation, thereby limiting fibrotic and inflammatory processes, while testosterone may enhance TGF-β expression through oxidative and metabolic stress pathways [71][72]. Consequently, the stronger reduction of TGF-β in males at higher extract doses may reflect a dose-dependent suppression of excessive TGF-β activity required to restore pancreatic tissue balance. In females, endogenous estrogen may already exert partial regulation of TGF-β signaling, resulting in less dose-dependent variation. These findings highlight that hormonal differences may influence how antioxidant and anti-inflammatory compounds modulate fibrotic cytokines under diabetic conditions.

3.6. Histopathological of Pancreas

Histopathological study of rat pancreas (Figure 12) revealed that all pancreatic cells underwent necrosis due to inflammation triggered by elevated GDP resulting from STZ administration.

The necrosis observed in the pancreas of both male and female rats in the DM model was marked by atrophied islets of Langerhans with indistinct cell borders. As a result of degenerative processes, pancreatic acini cells decrease, cell nuclei start to disintegrate, and vacuolization forms. The pancreas of rats administered with *C. bonduc* at doses of 200, 400, and 600 mg/kg exhibited qualitative enhancements in pancreatic tissue, particularly at the 400 mg/kg dosage, which displayed more distinct islets of langerhans and a reduction in acini cells compared to the 200 mg/kg and 600 mg/kg doses. When rats were given 600 mg/kg of *C. bonduc*, their pancreatic histology showed necrosis again, but not as badly as when they were given 200 mg/kg of *C. bonduc*. It can be deduced that

different dosages of *C. bonduc* seed extract affect the the histology of the rat pancreas. Quantitative histopathological scoring based on treatment dosage is presented in Figure 13. The results show that pancreatic damage was most severe in the 200 mg/kg group (mean = 3.00 ± 0.85), while the 400 mg/kg group showed the best histological improvement (mean = 1.00 ± 0.00). Meanwhile, the 600 mg/kg group exhibited partial recovery (mean = 2.50 ± 0.52). Statistical analysis revealed a significant difference among treatment groups ($p < 0.001$). Quantitative evaluation based on sex differences is presented in Figure 14. Male rats had a slightly higher mean pancreatic damage score (2.44 ± 1.19) than females (1.89 ± 0.76), but the difference was not statistically significant ($p = 0.147$).

The stimulation of STZ will elevate the expression of inflammatory cytokines (TNF-α, IFN-γ, IL-1β) and result in the manifestation of elevated blood glucose levels. STZ is a nitrosurea that is harmful to pancreatic beta cells in particular. STZ induction will lead to DNA alkylation. DNA

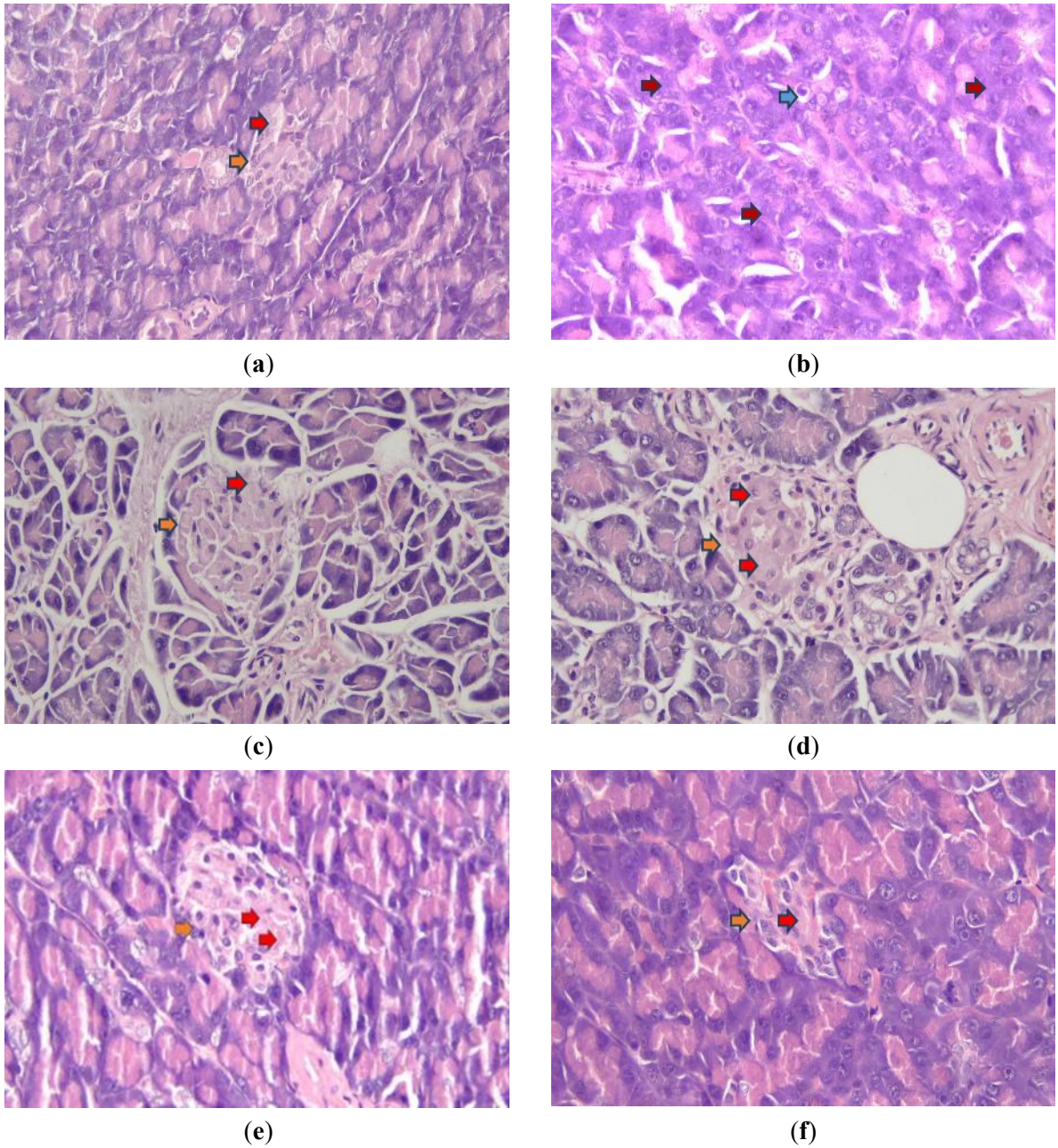


Figure 12. Histopathology results of the pancreas of DM model rats with H&E staining. (a) a2b1; (b) a1b1; (c) a2b2; (d) a1b2; (e) a2b3; (f) a1b3. Langerhans atrophy; Necrosis; Vacuolization;

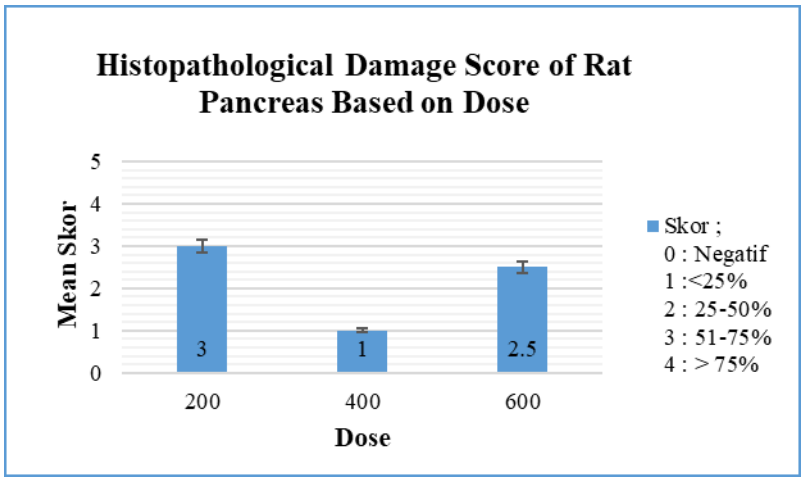


Figure 13. Bar graph of histopathological damage score of rat pancreas based on dose of ethanol extract of *Caesalpinia bonduc* seeds.

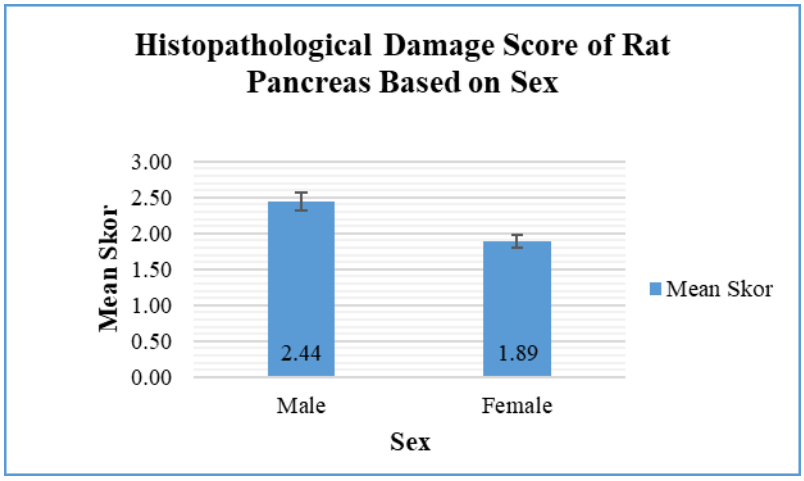


Figure 14. Bar graph of histopathological damage score of rat pancreas based on rat sex (male and female).

damage will activate the creation of the poly (ADP-ribose) synthase enzyme, which is an enzyme needed to repair DNA damage. This enzyme needs nicotinamide adenine dinucleotide (NAD) to work, which means that the amount of NAD in the cell goes down. The decrease in cellular NAD levels also results in a reduction of ATP levels, which halts the production and release of insulin, ultimately causing high blood sugar. Streptozotocin, functioning as a NO donor, can elevate ROS, including superoxide and hydroxyl radicals, as well as hydrogen peroxide. The reaction between NO and O_2, which can happen with free NO radicals or peroxynitrite compounds (ONOO), is very hazardous for pancreatic beta cells because it can destroy pancreatic DNA. STZ stops the Krebs cycle in the mitochondria and makes it harder for the mitochondria to consume oxygen, which limits the generation of ATP in the mitochondria. The enhanced reduction in ATP generation within mitochondria can augment the substrate availability for the enzyme xanthine oxidase, which catalyzes the formation of the active superoxide anion [73].

Diabetes development is associated with both quantitative losses (reduced β-cell mass or size) and qualitative damage (necrosis and degenerative changes) in pancreatic β-cells [35]. Combining STZ with NA frequently induces T2DM in animal models. STZ promotes the formation of reactive free radicals that damage cellular membranes, proteins, and DNA, ultimately impairing insulin secretion by β-cells within the islets of Langerhans [74]. Pancreatic β-cells are vital for glucose regulation because of their role in insulin production and release. Extensive β-cell injury or loss disrupts glucose homeostasis and leads to hyperglycemia [75][76]. Persistent high glucose levels accelerate the deterioration of β-cells,

reducing their numbers and impairing their insulin-producing capacity [77]. A growing body of studies highlights oxidative stress as a major contributor to acute inflammation, especially in the context of acute pancreatitis. Physiologically, ROS maintain essential roles in cellular regulation, influencing processes like proliferation, differentiation, migration, apoptosis, and the cell cycle [78]. The blood sugar level goes down because the pancreatic tissue grows better, which makes the pancreas make more insulin. Because of this, body cells can take in glucose from the blood and turn it into energy or store it as glycogen in the liver and muscles. This also fits with the correlation analysis that shows a strong link (correlation) between blood glucose levels and the variables MDA, TNF-α, and TGF-β. This suggests that lowering MDA, TNF-α, and TGF -β can also lower blood sugar levels.

These histopathological findings indicate that the 400 mg/kg dose provides an optimal therapeutic balance, effectively reducing pancreatic damage without causing the metabolic overload observed at higher doses such as 600 mg/kg. The milder pancreatic injury observed in female rats compared to males may be attributed to hormonal and metabolic differences. Estrogen exerts a protective effect against oxidative and inflammatory injury by modulating NF-κB signaling pathways and enhancing antioxidant enzyme activity, thereby mitigating β-cell damage [79][80]. Conversely, decreased testosterone levels in diabetic male rats may exacerbate oxidative and inflammatory responses, increasing the susceptibility of pancreatic tissue to STZ-induced injury [81][82]. Therefore, the optimization of dosage and the hormonal modulation associated with sex play a critical role in determining the extent of histological recovery of pancreatic tissue in diabetic models treated with *C. bonduc* extract.

4. CONCLUSIONS

The results of this study demonstrate that exposure to STZ-NA leads to a reduction in blood glucose levels through various pathways, including the utilization of antioxidants and anti-inflammatory compounds in the ethanol extract of *Caesalpinia bonduc* (L) Roxb seeds. In a rat model of type 2 diabetes, altering the quantity of ethanol extract from *C. bonduc* seeds markedly affected the levels of FBS, MDA, TNF-α, and TGF-β. They enhanced the microscopic appearance of the pancreas. Altering the sex of T2DM rat models had minimal impact on reducing FBS, MDA, TNF-α, and TGF-β levels, nor did it enhance pancreatic histology. The body derives advantages from these compounds as they function as antioxidants (IC_{50} = 55.361 ppm). The GC-MS results indicate that the extract contains five chemicals that are closely related. These chemicals are particularly significant to MDA, TNF-α, and TGF-β. The composition includes 6-octadecenoic acid, pregnenolone, (1*R*)-2α,5α,9α,10β-tetraacetoxytaxa-4(20)-11-dien-13-one, l-(+)-ascorbic acid 2,6-dihexadecanoate, and ethyl 9,12-octadecadienoate. This study indicates that the ethanol extract from *C. bonduc* seeds may possess antidiabetic properties. Further investigation is required to ascertain its efficacy as a treatment and its long-term safety.

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Conflicts of Interest

The authors declare no conflict of interest.

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DECLARATION OF GENERATIVE AI

During the preparation of this work, we are used DeepL pro to enhance English writing and Origin Lab for visualization the data.

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