

The Effect of Caffeic Acid Administration on the Antioxidant Status of the Seminal Vesicle in a High Fructose-Streptozotocin in a Diabetic Male Wistar Rat

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Abstract

Diabetes mellitus is a metabolic disease associated with oxidative stress, which impairs the histoarchitecture of seminal vesicles and impairs male reproductive activity. The aim of the study was to examine the protective properties of caffeic acid on diabetes-induced injury in male Wistar rat seminal vesicles. Twenty (20) adult rats were utilized with twenty controls having normal glucose levels and ten rats having diabetes induced by a single intraperitoneal injection of streptozotocin (50 mg/kg). A digital glucometer was used to measure the blood glucose levels 48 hours after induction, and a diagnosis of diabetes was made at a level of greater than 250 mg/dl, when the glucose level was found to be at fasting levels. The experimental groups (experimental, control, fructose-streptozotocin (FRUC-STZ), fructose-streptozotocin combined with caffeic acid (FRUC-STZ + CA)) were assigned randomly to the animals. The diabetic groups were fed with 10% fructose solution and caffeic acid (50 mg/kg) was given orally. Control group was treated with citrate buffer. The body weight and the level of blood glucose were monitored during the study. At the conclusion of the experimental period, animals were euthanized, and blood samples were taken to be tested by hormonal and biochemical analyses, and seminal vesicles were taken to be studied by a histological technique. Findings indicated that diabetic rats had high blood glucose levels, low insulin levels, and severe distortion of seminal vesicle structure. Nonetheless, these changes were greatly reduced upon treatment using caffeic acid to restore antioxidant status and promote tissue morphology. The results indicate that caffeic acid alleviates oxidative stress, improves the insulin activity, and maintains seminal vesicle integrity in diabetic circumstances.

Keywords: Caffeic Acid, Seminal Vesicles, Diabetes Mellitus, Antioxidants, STZ, Blood Sugar.

Introduction

Diabetes mellitus (diabetes) is a chronic metabolic condition that results from insufficient insulin production or a failure of insulin to work as it should (Ukwenya et al., 2020; Knight et al., 2017). Generation of free radicals, due to glucose oxidation, has been constantly

linked to diabetes mellitus (Celik et al., 2008). Diabetes mellitus is today one of the most common metabolic diseases in all age groups in the world, according to Victor et al., 2012. Additionally, it has been reported to adversely affect male reproductive and sexual functions in both humans and experimental animals (Yonezawa et al., 2009). Diabetes mellitus is becoming a serious health problem especially among reproductive age men. The endocrine dysfunction and worsening of sexual dysfunction and interference with functional activity of male accessory glands, especially the seminal vesicles, can disrupt male fertility in diabetes Mellitus (Frost et al., 2015; Ibrahim et al., 2015; Khimmaktong et al., 2019). In turn, this is likely to affect male fertility and reproductive capacity in the event of dysfunction of the seminal vesicles (Tsounapi et al., 2017).

The current therapeutic strategies against diabetes mellitus include the use of oral hypoglycemic agents, exogenous insulin administration, transplantation and xenotransplantation. Such treatment approaches, however, are not yet enough to get the disease under control or even cured (Adeyemi et al., 2014; Baynes, 2015; Baz et al., 2016). Although many studies have been performed to treat diabetes mellitus, an effective and safe therapeutic intervention has not yet been discovered. Diabetes-related oxidative stress also has been reported to cause structural and functional changes in the seminal vesicles, which could be a factor in male sterility (Al-Awar et al et al., 2016; Ashawal et al., 2015; Jia et al., 2019). Hence, the aim of this study is to examine therapeutic strategies that reduce the damage to the seminal vesicle of Wistar rats while not causing additional injury. Specifically, the present study investigates the effect of caffeic acid on body weight, blood glucose concentration, insulin activity, antioxidant status and histoarchitecture of the seminal vesicles in diabetic rats induced by fructose-streptozotocin (FRUC-STZ). The total thiol and protein thiol levels were used to measure antioxidant status, and hematoxylin and eosin (H&E) staining techniques were used to calculate the histological alterations. Caffeic acid has been shown to have antidiabetic effects on streptozotocin induced diabetic rats in previous investigations (Cheng et al., 2003; Hsu et al., 2000; Okunta et al., 2005). Caffeic acid, which is present in large amounts in coffee, fruits, legumes, olives, potatoes, carrots, grapes and plant resins, has demonstrated the most powerful antioxidant, antimutagenic, anticancer, antihyperglycemic and antidiabetic activity among the phenolic compounds present in food of natural origin (Agunloye et al., 2019; Birkova, 2020; Zahran et al., 2015; Okutan et al., 2005). It has also been reported that it inhibits carcinogenesis process and it is thought to play a role in the prevention of type 2 diabetes mellitus (Baynes, 2015; Baz et al., 2016; Herr et al., 1967; Mishra et al., 2024).

However, very little information is available regarding the precise effects of caffeic acid on the antioxidant status and/or histological structure of fructose-streptozotocin-induced diabetic models of seminal vesicles. In particular, the mechanisms of caffeic acid modulating total thiol and protein thiol levels in seminal vesicle tissues under oxidative stress conditions created by FRUC-STZ have not been well understood. The lack of understanding about the possible role of caffeic acid in preventing diabetes-associated SV dysfunction warrants further research.

Statement of the Problem

Diabetes mellitus (DM) is a chronic metabolic disease defined by high blood glucose levels and/or elevated levels of free radicals, which are generated during glucose metabolism causing increased oxidative stress (Celik et al., 2008; Victor et al., 2012). Diabetes mellitus has been shown to have harmful effects on the male reproductive system; a few studies showed that diabetes mellitus caused structural and functional changes in male reproductive organs (Yonezawa et al., 2009; Frost et al., 2015; Ibrahim et al., 2015; Khimmaktong et al., 2019). The ability of oxidative stress in diabetes to lead to tissue degeneration, alteration of secretory activity and histoarchitectural distortion of the seminal vesicles (SV) could also play a role in male infertility (Al-Awar et al et al., 2016; Ashawal et al., 2015; Jia et al., 2019; Tsounapi et al., 2017).

Diabetic therapies (including insulin therapy and oral hypoglycemic agents) currently in use are primarily aimed at glycemic control, and often engender side effects, high costs, and fail to completely prevent diabetes-associated reproductive problems (Adeyemi et al., 2014; Baynes, 2015; Baz et al., 2016). Thus, there is a growing focus on natural bioactive compounds bearing antioxidant and anti-diabetic activities. Of these compounds, caffeic acid has attracted scientific interest with regard to its antioxidant, antihyperglycemic, antimutagenic and antidiabetic activities (Agunloye et al., 2019; Birkova, 2020; Cheng et al., 2003; Zahran et al., 2015; Okutan et al., 2005). The previous studies showed that caffeic acid has hypoglycemic and anti-oxidative effects in streptozotocin induced diabetic models (Cheng et al., 2003; Hsu et al., 2000; Okutan et al., 2005).

Although the pharmacology results have been reported, little empirical evidence has been reported about the specific effect of caffeic acid on the seminal vesicles of fructose-streptozotocin (FRUC-STZ)-induced diabetic rats. Furthermore, how caffeic acid affects biomarkers of antioxidants (total thiol content and protein thiol content) in diabetic conditions in the tissues of the seminiferous vesicles is not very clear. Additionally, little is known about whether caffeic acid can maintain or repair the histointegrity of seminal vesicles compromised by oxidative stress caused by FRUC-STZ.

Thus, the main problem in the present study was the inadequate scientific information regarding the protective effect of caffeic acid against oxidative and histopathological changes in the seminal vesicles of FRUC-STZ diabetic rats. This study aims to close this gap of knowledge by examining the effect of caffeic acid on antioxidant status, glycemic regulation and histoarchitecture of the seminal vesicles of diabetic Wistar rats.

Objectives of the Study**General Objective**

The general objective of this study is to investigate the protective and therapeutic effects of caffeic acid on seminal vesicle alterations induced by fructose-streptozotocin (FRUC-STZ)-induced diabetes mellitus in Wistar rats.

Specific Objectives

The specific objectives of this study are to:

1. Determine the effect of caffeic acid on the body weight of FRUC-STZ-induced diabetic Wistar rats.
2. Evaluate the effect of caffeic acid on blood glucose levels in FRUC-STZ-induced diabetic Wistar rats.
3. Assess the effect of caffeic acid on insulin levels in FRUC-STZ-induced diabetic Wistar rats.
4. Investigate the effect of caffeic acid on antioxidant status by measuring total thiol and protein thiol levels in the seminal vesicles of FRUC-STZ-induced diabetic Wistar rats.
5. Examine the effect of caffeic acid on the histoarchitecture of seminal vesicles in FRUC-STZ-induced diabetic Wistar rats using hematoxylin and eosin (H&E) staining techniques.
6. Evaluate the potential protective role of caffeic acid against oxidative stress-induced damage in the seminal vesicles of diabetic Wistar rats.

Research Questions

This study seeks to answer the following research questions:

1. What effect does caffeic acid have on the body weight of FRUC-STZ-induced diabetic Wistar rats?
2. How does caffeic acid influence blood glucose levels in FRUC-STZ-induced diabetic Wistar rats?
3. What effect does caffeic acid have on insulin levels in FRUC-STZ-induced diabetic Wistar rats?
4. Does caffeic acid improve antioxidant status by altering total thiol and protein thiol levels in the seminal vesicles of FRUC-STZ-induced diabetic Wistar rats?
5. Can caffeic acid ameliorate histopathological alterations in the seminal vesicles of FRUC-STZ-induced diabetic Wistar rats?
6. To what extent does caffeic acid protect seminal vesicle tissues against oxidative stress associated with diabetes mellitus?

Directional Research Hypotheses

H₁: Caffeic acid administration significantly reduces blood glucose levels in fructose-streptozotocin (FRUC-STZ)-induced diabetic Wistar rats compared with untreated diabetic rats.

H₂: Caffeic acid administration significantly improves body weight in FRUC-STZ-induced diabetic Wistar rats compared with untreated diabetic rats.

H₃: Caffeic acid administration significantly increases insulin levels in FRUC-STZ-induced diabetic Wistar rats compared with untreated diabetic rats.

H₄: Caffeic acid administration significantly increases total thiol levels in the seminal vesicles of FRUC-STZ-induced diabetic Wistar rats compared with untreated diabetic rats.

H₅: Caffeic acid administration significantly increases protein thiol levels in the seminal vesicles of FRUC-STZ-induced diabetic Wistar rats compared with untreated diabetic rats.

H₆: Caffeic acid administration significantly reduces oxidative stress-induced damage in the seminal vesicles of FRUC-STZ-induced diabetic Wistar rats.

H₇: Caffeic acid administration significantly improves the histoarchitecture of seminal vesicles in FRUC-STZ-induced diabetic Wistar rats by restoring normal epithelial organization and reducing tissue degeneration compared with untreated diabetic rats.

H₈: The protective effects of caffeic acid on seminal vesicle tissues are associated with enhanced antioxidant defense mechanisms in FRUC-STZ-induced diabetic Wistar rats.

Conceptual Framework

The conceptual scheme of this research is founded on the connection between the diabetes mellitus induced by fructose-streptozotocin (FRUC-STZ), the oxidative stress pathways and the antioxidant protective mechanism of caffeic acid in seminal vesicle tissues. The diabetes induced by FRUC-STZ is a widely used experimental model of type 2 diabetes mellitus, as it mimics both the metabolic characteristics of type 2 diabetes mellitus in humans as well as insulin resistance caused by fructose and β -cell dysfunction caused by streptozotocin (Srinivasan et al., 2005; Skovso, 2014). This metabolic imbalance leads to chronic hyperglycaemia, decreased insulin function, and increased reactive oxygen species (ROS) production (American Diabetes Association, 2023).

Diabetic hyperglycaemia leads to oxidative stress via multiple biochemical pathways, such as glucose auto-oxidation, the formation of advanced glycation end-products (AGEs), lipid peroxidation and mitochondrial dysfunction (Giacco & Brownlee, 2010). An excess of ROS production causes depletion of antioxidant biomarkers like total thiol and protein thiol groups (Betteridge, 2000). The loss of these antioxidants leads to damage of the cellular membranes, inflammatory reactions and degeneration of reproductive tissues such as the seminal vesicles (Aitken & Roman, 2008). Thus, oxidative stress can affect the secretory function of the seminal vesicles, and can lead to changes in the tissue histoarchitecture, which are all detrimental to male reproductive function (La Vignera et al., 2012).

Caffeic acid (CA), a naturally occurring polyphenolic compound, is known for its strong antioxidant and antihyperglycemic activity. It is found in high amounts in coffee, fruits, vegetables and some medicinal plants (Magnani et al 2014). It has been demonstrated that its antioxidant action is based on its free radical scavenging properties, its ability to inhibit lipid peroxidation, its ability to chelate transition metals, and its ability to strengthen the body's antioxidant defense mechanisms (Touaibia & Guay 2011). Experimental studies have shown that caffeic acid possesses beneficial properties of improvement of glucose metabolism, decrease of oxidative stress and protection of tissues from injury caused by diabetes mellitus (Jayanathi et al., 2010; Oboh et al., 2015). Caffeic acid is predicted to slow

down the oxidative stress in the body, maintain insulin sensitivity, lower hyperglycemia and prevent histopathological damage to the tissues of the seminal vesicles in diabetic rats.

The antioxidant mechanism of caffeic acid may be represented conceptually as:

ROS+ Caffeic Acid → Reduced Oxidative Damage

Based on this, it is proposed that diabetes induced by FRUC leads to an increase in oxidative stress and degeneration of the tissues of the seminal vesicles, while caffeic acid may help to resolve the effects of diabetes by its antioxidant activity and the ability to restore the redox balance of cells. The study then aims to determine the efficacy of the antioxidant markers (total thiol and protein thiol levels) and to restore normal histoarchitecture of the seminal vesicles in diabetic Wistar rats.

Literature Review

Diabetes Mellitus, Oxidative Stress, and Male Reproductive Dysfunction

Diabetes Mellitus is a chronic metabolic disease characterized by persistent hyperglycemia resulting from impaired insulin secretion, insulin resistance, or both. Recent studies have established that oxidative stress plays a central role in the pathophysiology and progression of diabetes mellitus and its associated complications. Hyperglycemia promotes excessive production of reactive oxygen species (ROS) through glucose auto-oxidation, mitochondrial dysfunction, activation of the polyol pathway, and formation of advanced glycation end-products (AGEs), thereby overwhelming endogenous antioxidant defense systems (Dena et al., 2025; Boštjan Martinc, 2026).

Oxidative stress has increasingly been implicated in male reproductive dysfunction associated with diabetes mellitus. Recent evidence indicates that ROS-mediated damage affects reproductive hormones, sperm integrity, testicular architecture, and accessory reproductive glands (Xu et al., 2021). Diabetes-related oxidative stress has been shown to impair male fertility by inducing lipid peroxidation, DNA fragmentation, mitochondrial dysfunction, and apoptosis in reproductive tissues (Kowalczyk, 2022).

Although many investigations have focused on testicular and sperm abnormalities, comparatively limited attention has been directed toward the seminal vesicles despite their important contribution to seminal fluid production and reproductive function. Existing literature suggests that oxidative stress may disrupt seminal vesicle secretory activity and tissue organization, thereby contributing to reduced fertility potential in diabetic males. However, detailed mechanistic studies evaluating antioxidant modulation within seminal vesicle tissues remain insufficient.

FRUC-STZ-Induced Diabetes Model in Experimental Research

The fructose-streptozotocin (FRUC-STZ) diabetic model is widely recognized as an effective experimental model for type 2 diabetes mellitus because it combines fructose-induced insulin resistance with streptozotocin-mediated pancreatic β -cell dysfunction. This model closely reproduces several metabolic and biochemical features of human type 2 diabetes, including chronic hyperglycemia, oxidative stress, and impaired insulin signaling.

Seminal studies by Srinivasan and later investigations established the suitability of this model for evaluating antidiabetic and antioxidant therapies in experimental animals (Muhammed et al., 2021).

Recent studies continue to validate the relevance of oxidative stress biomarkers in FRUC-STZ-induced diabetic conditions. Oxidative imbalance in this model is commonly characterized by depletion of antioxidant molecules, including glutathione, thiol-containing proteins, catalase, and superoxide dismutase (Boštjan Martinc, 2026). However, while oxidative stress mechanisms in diabetic complications have been extensively studied in organs such as the liver, kidneys, heart, and testes, there remains inadequate information regarding oxidative injury specifically within seminal vesicle tissues in FRUC-STZ diabetic models.

This limitation represents a major gap in the literature because seminal vesicles play a crucial role in reproductive physiology, and their dysfunction may significantly contribute to infertility associated with diabetes mellitus.

Antioxidant Properties of Caffeic Acid

Caffeic Acid is a naturally occurring hydroxycinnamic acid present in coffee, fruits, vegetables, legumes, and several medicinal plants. Over the last five years, scientific interest in caffeic acid has increased substantially because of its antioxidant, anti-inflammatory, antihyperglycemic, and cytoprotective properties. Recent reviews have demonstrated that caffeic acid scavenges free radicals, inhibits lipid peroxidation, modulates inflammatory signaling pathways, and improves cellular antioxidant defense mechanisms (Pavlikova, N. 2023).

Systematic evidence further suggests that caffeic acid and its derivatives exert beneficial effects in experimental diabetes by reducing hyperglycemia, improving insulin sensitivity, and attenuating oxidative stress. Studies summarized in recent systematic reviews indicate that caffeic acid enhances endogenous antioxidant systems and reduces inflammatory cytokine activity in diabetic tissues (Iman et al., 2023).

Furthermore, recent literature has highlighted the therapeutic potential of flavonoids and phenolic compounds in managing male reproductive disorders. Natural antioxidants have been shown to improve reproductive tissue integrity by reducing oxidative damage and restoring redox balance (Mishra et al., 2024).

Contradictions and Gaps in Existing Literature

Despite growing evidence supporting the antioxidant and antidiabetic effects of caffeic acid, important inconsistencies and research gaps remain unresolved. First, most recent studies evaluating caffeic acid have concentrated on systemic metabolic outcomes such as blood glucose regulation, hepatic protection, nephropathy, cardiomyopathy, and neuropathy (Iman et al., 2023). Comparatively few studies have investigated its effects on male reproductive accessory glands, particularly the seminal vesicles.

Second, although oxidative stress has been identified as a major contributor to diabetic reproductive dysfunction, many investigations primarily assess general antioxidant enzymes without specifically evaluating thiol-based antioxidant biomarkers such as total thiol and protein thiol concentrations in seminal vesicle tissues. Thiol-containing compounds are critical components of cellular redox regulation, yet their modulation by caffeic acid in FRUC-STZ-induced diabetic seminal vesicles remains poorly understood.

Third, contradictions exist regarding the extent to which antioxidant therapy alone can reverse diabetes-induced reproductive tissue damage. Some studies report significant histological recovery following antioxidant treatment, whereas others suggest only partial restoration of tissue architecture due to persistent metabolic disturbances and chronic inflammation (Mishra et al., 2024). These inconsistencies indicate that the protective mechanisms of antioxidants in diabetic reproductive tissues have not been fully clarified.

Another major limitation in the literature is the scarcity of studies simultaneously evaluating biochemical antioxidant markers and histopathological outcomes within the same experimental framework. Many studies assess either oxidative stress parameters or tissue histology independently, thereby limiting comprehensive understanding of the relationship between antioxidant restoration and structural tissue recovery.

Therefore, the present study is designed to address these identified gaps by investigating the effects of caffeic acid on antioxidant status specifically total thiol and protein thiol levels and seminal vesicle histoarchitecture in fructose-streptozotocin-induced diabetic Wistar rats. By integrating biochemical and histological assessments, this study aims to provide clearer mechanistic insight into the protective role of caffeic acid against diabetes-induced seminal vesicle damage.

Materials and Methods

Wistar rats, injections (1 ml and 5 ml), insulin syringe, regular saline, petri plates, simple specimen tubes, laboratory attire, needles, conical flask, dissecting sets, cotton wool, dissecting board, weighing balance, hand gloves, blades, slides, animal cages, EDTA bottles, cannula, cold centrifuge, Accu Check glucometer, analytical balance, methylated spirit, and an electric kitchen scale. Fructose, caffeic acid, streptozocin, etc. (Sigma Chemical Co., USA).

Animal Care and Management

The Department of Anatomy at Ekiti State University in Ekiti, Nigeria, provided twenty males mature Wistar rats, weighing 236 ± 38 g and with a fasting blood sugar level of 64 ± 15 mg/dl. They were brought to the animal house of the anatomy department at the Federal University of Technology Akure in Ondo state, Nigeria, where they were housed in polycarbonate cages with a controlled ambient temperature of about 25°C and a 12-hour light-dark cycle. For two weeks, all of the rats were acclimated and given free access to tap water and regular rat chow. The ethics approval reference number is not available within my reach.

Experimental Design

Twenty rats were used in this experiment, of which 2 groups each containing 5 rats were considered the Control group and Caffeic acid (CA) group respectively while the remaining 10 rats were used for experimental diabetes by the method of giving 10% fructose solution ad libitum for two weeks followed by a single intraperitoneal injection of 50mg/kg of streptozotocin (STZ). Diabetes was confirmed over 3 days by fasting glucose levels > 250mg/dl after which the rats were divided into two groups of 5: Fructose-Streptozotocin (FRUC-STZ) and Fructose-Streptozotocin + Caffeic Acid (FRUC-STZ +CA). This study was performed following the Guide for the care and use of laboratory animals, approved by the FUTA Institutional Review Board. The rats were randomly divided into the following experimental groups:

- Control = 2ml citrate buffer
- Fructose-Streptozotocin (FRUC-STZ) = 10% fructose solution (ad libitum) + 50mg/kg body weight STZ for 2 weeks
- Fructose-Streptozotocin + Caffeic Acid (FRUC-STZ+ CA) = 2 weeks 10% fructose solution ad libitum + 50mg/kg body weight STZ + 50mg/kg body weight CA
- Caffeic Acid (CA) = 50mg/kg body weight CA

Caffeine acid (50mg/kg) was dissolved in distilled water and given orally. Normal control group was also treated with citrate buffer by the mouth. Euthanasia was performed 24 hours after the last dose with the serum being collected for biochemical assay while the whole seminal vesicles collected for histological evaluation.

Power Analysis

A priori power analysis was performed to establish the minimum sample size needed for the study with α of 0.1, 80% of statistical power and large effect size of 0.8. The analysis showed that 5 animals per group was enough to achieve a statistically significant difference between the experimental groups (this is a post-hoc power calculation with assumed effect sizes stated).

Induction of Diabetes

The animals were administered 10% fructose solution at will for two weeks following a two-week acclimatization phase. Following an overnight fast on the final day of administering fructose, the rats received a single intraperitoneal injection of STZ on the fifteenth day at a 50 mg/kg body weight dosage. The STZ was then dispersed in 0.1M citrate buffer at pH 4.5. The 0.1M citrate buffer was made by dissolving 2.94g of sodium citrate and 2.1g of citric acid in 100ml in distilled water. The pH was buffered to 4.5 using sufficient volumes of pure NaOH/HCl. Using the proper glucometer strips and an Accu glucometer, blood samples from the dorsal tail vein were tested for fasting blood glucose 48 hours after diabetes induction. The blood glucose level of the diabetic rats used in this

investigation had to be at least 250 mg/dl. After the diagnosis of diabetes was confirmed, treatment started. Every three days, the body weight and glucose levels measurement was carried out on the animals.

Ethics statement: The study was conducted in accordance with the regulations for the use and care of laboratory animals with permission from the FUTA Institutional Review Board. Furthermore, all animal methods used in this study were authorized by the Departmental Committee on the Use and Care of Animals and complied with the guidelines for animal research as outlined by the Declaration of Helsinki. The animals were appropriately put to death after 14 days of treatment.

Seminal Vesicle Tissue Histology: Hematoxylin and Eosin

The rats' whole seminal vesicles were gathered, preserved in neutral buffered formalin for 48 hours, and then transferred to 70% alcohol for dehydration. After passing through xylene and 90% and 100% alcohol for varying lengths of time, the tissues are placed in molten paraffin for an hour at 65° in each oven to allow for infiltration. A rotary microtome was used to implant the tissues and cut serial slices of five microns. Following the selection of tissues containing albumenized slides and a two-minute drying interval on heated plates, the slides had been dewaxed utilizing xylene and subjected to three rounds of alcohol (absolute, 90%, 70%, and 50%) before being immersed in water for five minutes. Following hematoxylin and eosin staining, slides had been placed in DPX and captured on camera using a Leica DM750 microscope.

Preparing Serum from Blood Samples

Cardiac punctures were used to draw blood, which was then placed in sterile plain bottles and left to clot for two hours at room temperature. To acquire the sera, centrifugation was performed at 2500 rpm for 20 minutes using a C-Golden-wall centrifuge (China). After aspirating the sera from the various samples using micropipettes into the corresponding sterile plastic sample bottles with labels, the samples were promptly chilled for use in the biochemical experiment: Testosterone, total thiol, protein thiol, and insulin. No TNF- α was done in biochemical assay.

Biochemical Assay

Commercial kits were used for all biochemical tests. Commercial kits were used for the insulin assay. An ELISA (Enzyme Linked Immunosorbent Assay) kit from RayBioTM was used to measure insulin; an ELISA kit from Sigma-Aldrich was used to measure protein thiol; and an ELISA kit from Cell Biolabs was used to measure total thiol. The Sigma-Aldrich ELISA kit was used for the colorimetric assay.

Statistical Analysis

The values were expressed as mean $\pm$ S.E.M. (standard error of mean). The Tukey's test for multiple comparison and one-way analysis of variance (ANOVA) were used to determine

the statistical significance of the mean and standard error of mean difference at $p < 0.1$. Although the difference did not reach statistical significance ($p < 0.05$), a trend toward improvement was observed. Effect size measures includes eta-squared for ANOVA comparisons and cohen's d where appropriate.

Results

Body Weight

Table 1 presents total body weight data. When comparing the initial and end body weights, the FRUC-STZ group exhibits the greatest decline. The baseline and end body weights of the treatment and control groups varied very little.

Table 1: shows the experimental animals' initial and final body weight changes over a 14-day period. * Indicates a substantial drop at $p < 0.1$ to the Control, FRUC-STZ+CA, and CA groups; values are mean $\pm$ SEM; N = 5.

Groups	Initial weight (g)	Final weight (g)	Weight difference (g)
Control	195.7 $\pm$ 12.6	204.7 $\pm$ 16.7	9.0 $\pm$ 4.1
FRUC-STZ	206.8 $\pm$ 12.8	167.3 $\pm$ 8.2*	39.5 $\pm$ 1.6
FRUC-STZ+CA	213.6 $\pm$ 9.3	210.2 $\pm$ 11.7	3.4 $\pm$ 2.4
CA	228.8 $\pm$ 12.7	209.6 $\pm$ 10.0	19.2 $\pm$ 2.7

Total Blood Sugar

The animals' total blood glucose levels are shown in Table 2. In contrast to the FRUC-STZ+CA group (116.3 $\pm$ 38.6 mg/dL), which had final blood glucose levels closer to the control group (76.8 $\pm$ 3.6 mg/dL) and CA group (72.6 $\pm$ 3.7 mg/dL), the FRUC-STZ group had a high average final glucose level in the diabetes range (234.0 $\pm$ 32.6 mg/dL).

Table 2: shows the experimental animals' initial and ultimate blood glucose levels after a 14-day period. N = 5. * Indicates a significant increase at $p < 0.1$ in comparison to the Control group; values represent mean $\pm$ SEM. # indicates a significant decrease in comparison to the FRUCTOSE/STZ group at $p < 0.1$.

Groups	Initial glucose (mg/dL)	Final glucose (mg/dL)	Glucose difference (mg/dL)
Control	47.5 $\pm$ 2.9	76.8 $\pm$ 3.6 #	29.3 $\pm$ 0.7
FRUC-STZ	306.3 $\pm$ 16.5 *	234.0 $\pm$ 32.6	72.3 $\pm$ 16.1
FRUC-STZ+CA	340.3 $\pm$ 31.0 *	116.3 $\pm$ 38.6 #	224.0 $\pm$ 7.6
CA	57.2 $\pm$ 2.0	72.6 $\pm$ 3.7 #	15.4 $\pm$ 1.7

Hormonal Assay: Insulin

Figure 1 presents insulin data. When compared to the Control (1.112 $\pm$ 0.032) and CA (0.979 $\pm$ 0.728) groups, the FRUC-STZ+CA (1.120 $\pm$ 0.075) group has the highest insulin levels,

whereas the FRUC-STZ (0.973 ± 0.74) group has the lowest. The groups did not differ significantly ($p < 0.1$).

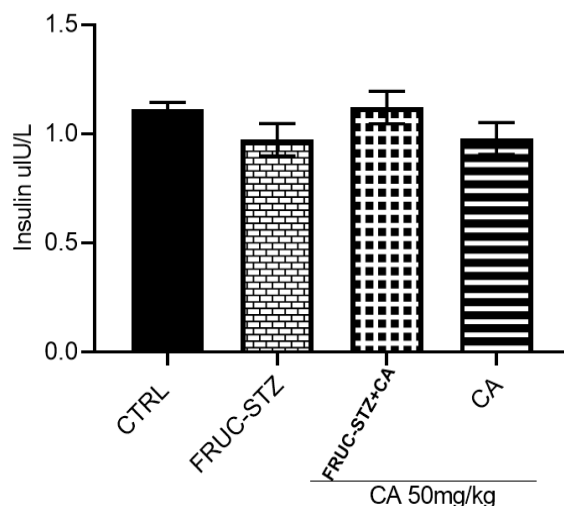
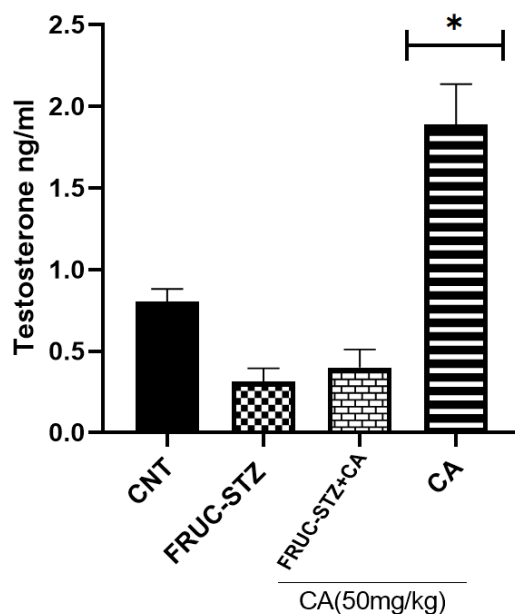


Figure 1: Insulin for groups undergoing experiments. The values are mean $\pm$ SEM. Each group has N = 5.

Testosterone

Figure 2 displays information on testosterone. Testosterone levels in the control group (0.31 ± 0.08) are lower than those in the other groups, with no discernible difference. The FRUC-STZ group (0.39 ± 0.11), the FRUC-STZ and Caffeic acid group (0.80 ± 0.07), and the Caffeic group (1.89 ± 1.42) all have considerably different levels of insulin.



Figure

2:

Caffeic acid's effects on testosterone levels in normal and experimental rats with STZ-induced diabetes. * Indicates a significant difference at $p < 0.1$ between the CNT, FRUC-STZ, and FRUC-STZ + CA groups.

Biochemical Assay

Total Thiol

Figure 3 shows the overall thiol concentrations. The total thiol of the Control only group (1.060 ± 0.104) is significantly higher ($p < 0.1$) than that of the FRUC-STZ (0.584 ± 0.028), FRUC-STZ + CA (0.694 ± 0.036), and CA (0.778 ± 0.044). Though there was no statistically notable difference at $p < 0.1$, the diabetes group gave a value lowest in comparison to the control, treatment (FRUC-STZ+CA), and CA groups.

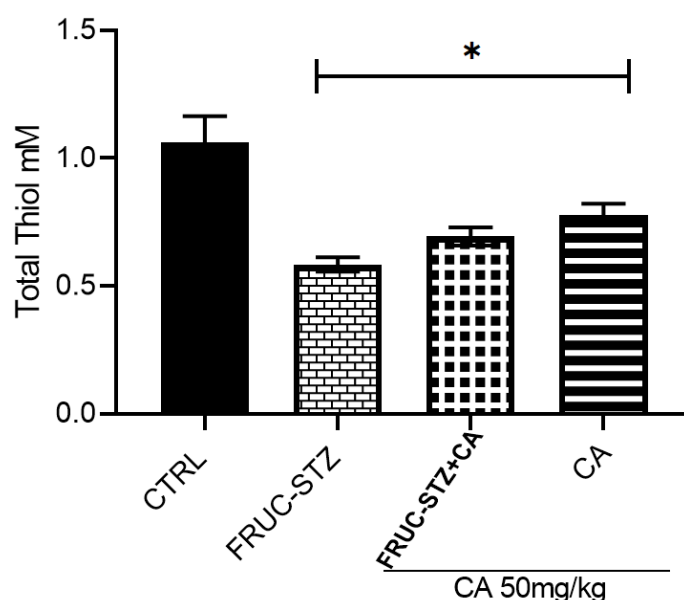


Figure 3: Total thiol in serum for the experimental groups. The values are mean $\pm$ SEM. Each group has N = 5. * Indicates a significant difference from the control group at $p < 0.1$.

PROTEIN THIOL

Figure 4 shows the amounts of protein thiol in the serum. Although there was no statistically significant difference at $p < 0.1$, the diabetes group had the lowest value in comparison to the control, treatment (FRUC-STZ+CA), and CA groups. In comparison to the FRUC-STZ (0.492 ± 0.082), FRUC-STZ + CA (0.631 ± 0.058), and CA (0.596 ± 0.095) groups, the Control group (0.652 ± 0.145) displayed the greatest values.

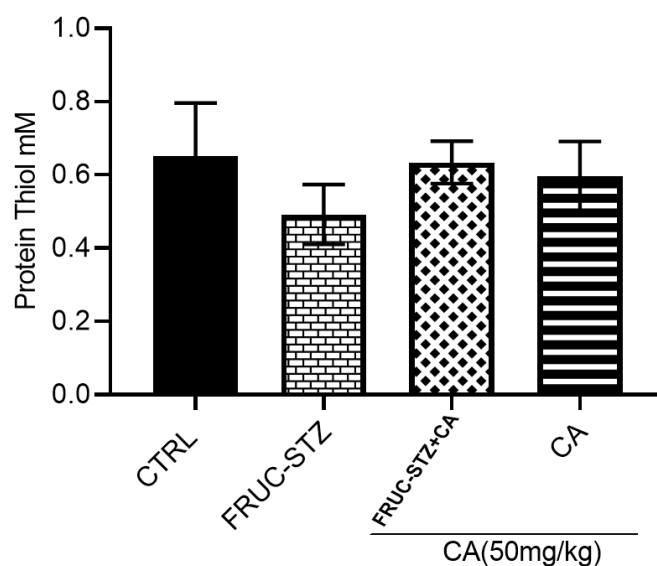


Figure 4: The experimental groups' serum protein thiol. The values are mean $\pm$ SEM. Each group has N = 5.

Histology

Hematoxylin and Eosin – Figure 5a-5d shows the representative photomicrographs of the seminal vesicle, which is bordered by a pseudostratified columnar epithelium, appears to the Control group as thin, densely branched mucosal folds inside honeycombed saccules. These folds join to create asymmetrical chambers that exchange information with a large central lumen that contains a uniform, translucent secretion [24]. Compared to the control group, the smooth muscles at the gland's saccular dilatation are disrupted in the FRUC-STZ or diabetic group. There are few fluid deposits and the lumina is expanded. The attenuation of smooth muscle fibers disrupts the connective tissue stroma. The basement membrane of the epithelium thickens. The therapy group's (FRUC-STZ+ CA) representative micrographs revealed distinct layers of smooth muscle in the connective stroma, embedded saccules with well-lined pseudostratified columnar epithelium, and a moderate lumen resembling that of the control group. The CA group's seminal vesicles had the same histo-architecture as the Control group, appearing as honeycombed saccules with a pseudostratified columnar epithelium lining thin, highly branching mucosal folds. These folds join to create asymmetrical compartments that exchange information with a large central lumen that contains a uniform, transparent secretion.

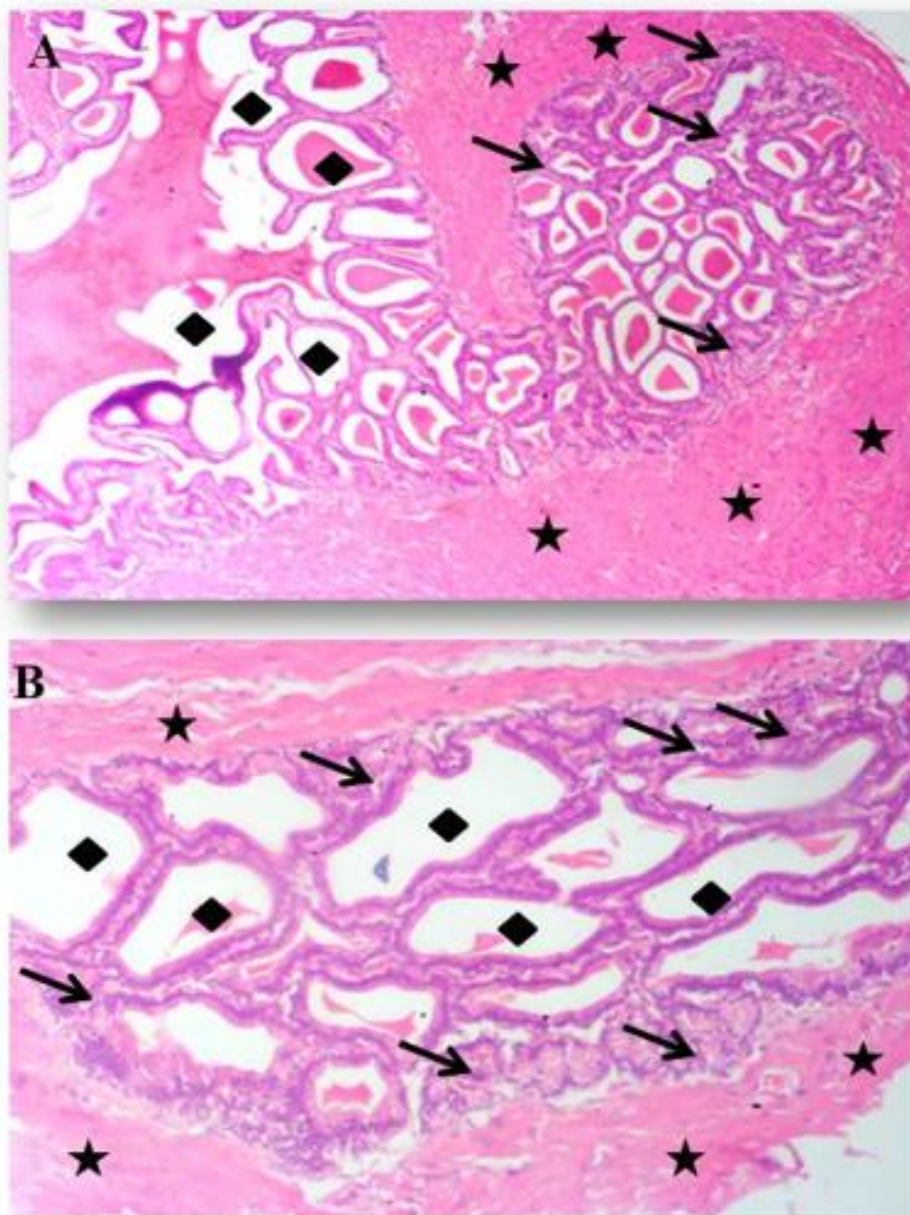


Figure 5a: Representative micrographs from the Control group. Stain: H and E
Slide A: Mag X40 and Slide B: Mag X100. Slides showing tissue with a well differentiated smooth muscles (stars), a normal epithelial tissue (arrows) and surrounding luminal spaces (diamonds).

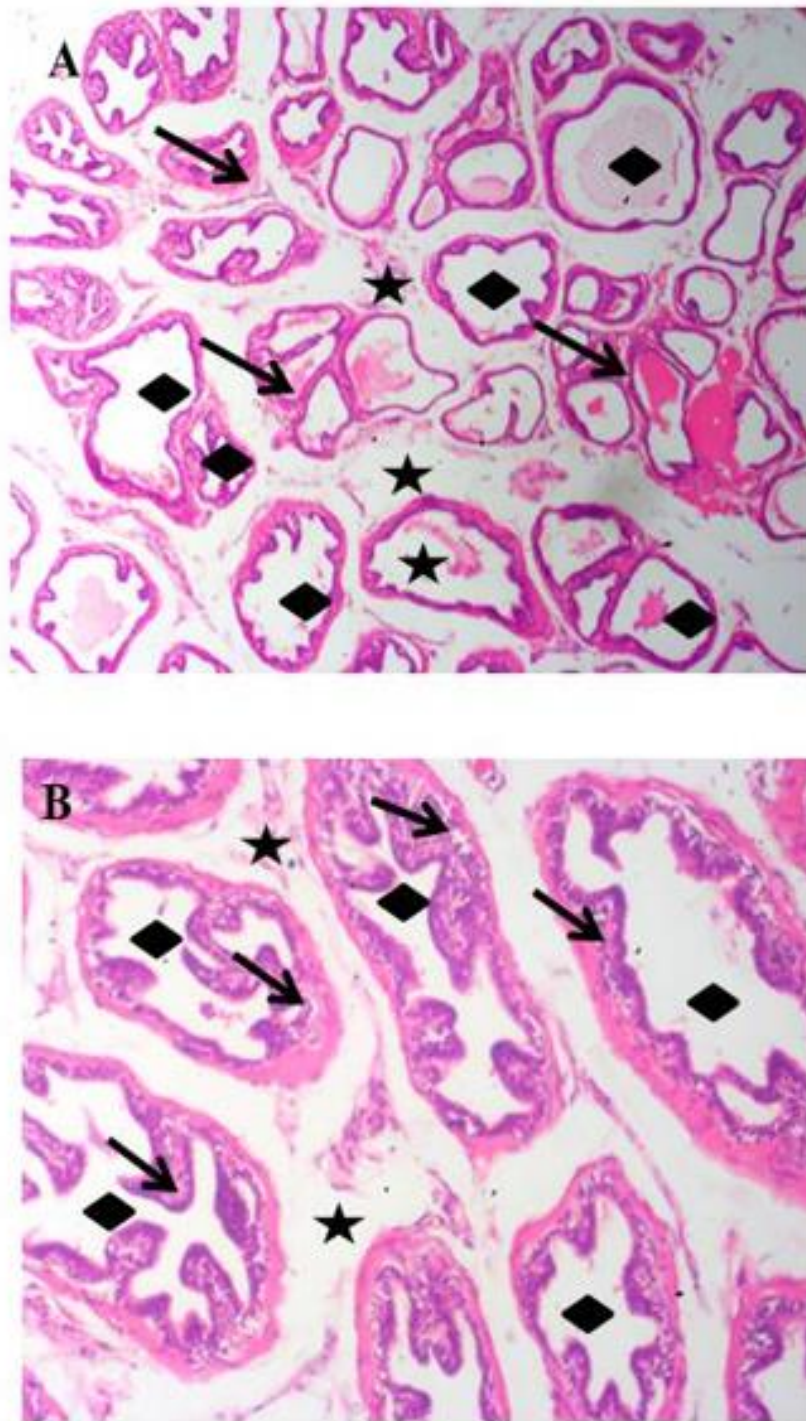


Figure 5b: Micrographs representative of the FRUC-STZ group. Stain: H and E Slides A and B show the Mag X40 and X100, respectively. Slides displaying shrinkage and degeneration of epithelial tissue (arrows), less secretion in the lumen and vacuolation (diamonds), and thinning or damage to the smooth muscles (stars).

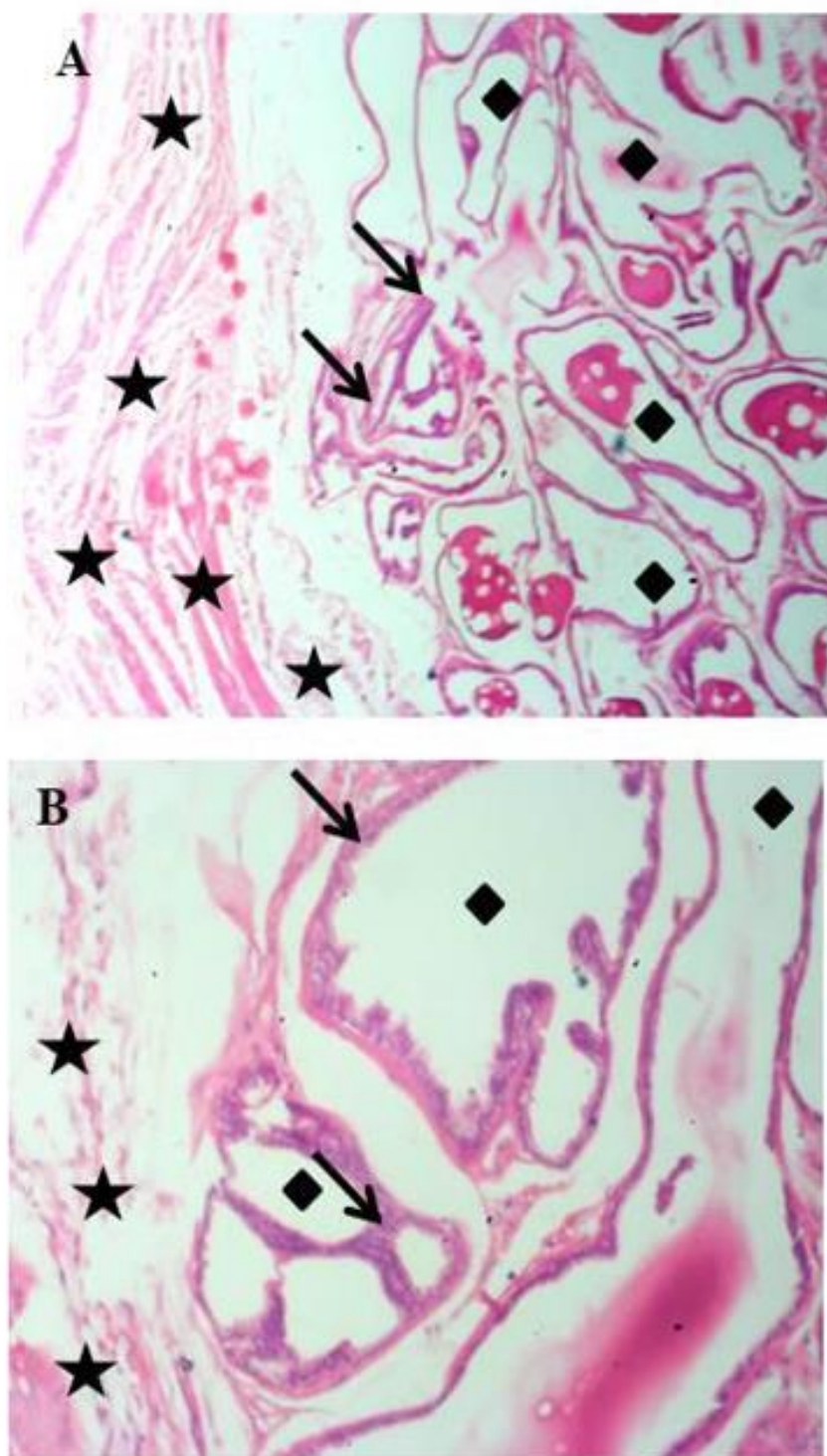


Figure 5c: The FRUC-STZ+ CA group's representative micrograph. Slide A: Mag X40; Slide B: Mag X100; Stain: H and E. Slides displaying smooth muscle eruptions (stars), well defined epithelial tissues (arrows), and a decrease in luminal gaps (diamonds).

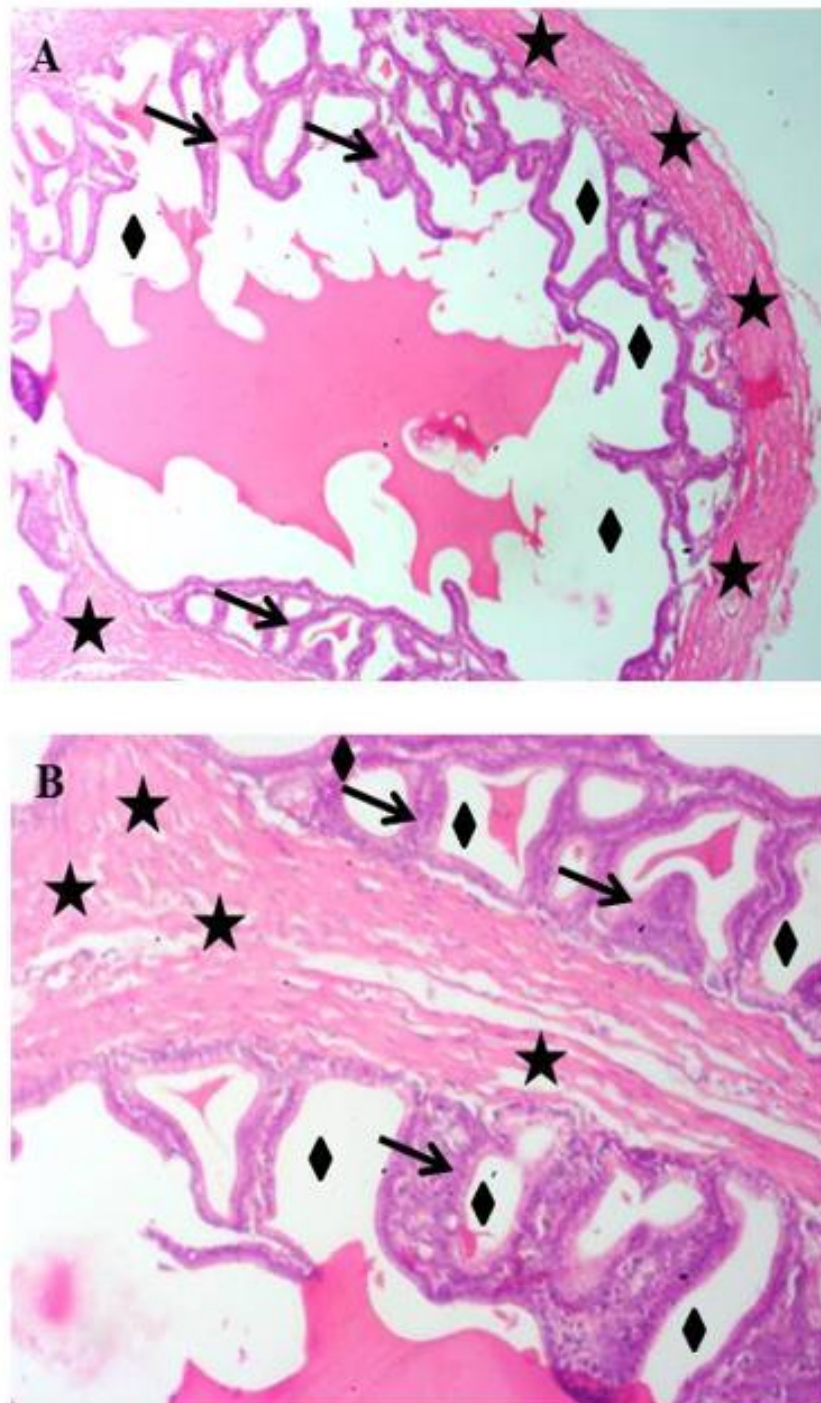


Figure 5d: Representative micrograph of the CA group. Stain: H and E. Slides A and B show the Mag X40 and X100, respectively. Slides with a surrounding luminal space (diamonds), normal epithelial tissue (arrows), and well-differentiated smooth muscles (stars).

Discussion

The proper development, upkeep, and secretory function of Peripheral sex organs in men are mostly controlled by hormones related to androgens (Kochakian, 1973). This study has demonstrated that the size and role of the seminal vesicles are negatively impacted by diabetes mellitus. Diabetes caused the seminal vesicles to dramatically atrophy, as shown by the organ's markedly decreased weight and altered histological analysis. The organs' inability to secrete and contract a sufficient amount of seminal vesicular fluid containing the right components was caused by this atrophy (McGraw et al., 2015). Testosterone shortage, excessive oxidative stress, and muscle cell cytoplasmic shrinkage and total muscle layer condensation are all associated with irregular spasms of the seminal vesicular tissue caused by diabetes. This provides proof that oxidative stress brought on by diabetes plays a critical role in seminal vesicle dysfunction. We do not rule out the significant part testosterone plays in the seminal vesicles' dysfunction brought on by diabetes. Our results show that injection of caffeic acid can reduce hyper-contractility of the seminal vesicles by regulating tissue histology and significantly lowering oxidative stress indicators without affecting blood testosterone levels.

When examining how STZ-induced diabetes affected the seminal vesicles, Morrison et al. found that the organ's alterations might be caused by the regeneration and remodeling of the ends of sympathetic nerves that had been harmed in the initial stages of hyperglycemia (Morrison et al., 2006). The main secretory changes seen in pseudostratified tall columnar epithelial cells include microvesicular lipid droplets and certain characteristics of lipofuscin pigment granules. The unusually little amount of seminal vesicular fluid in the DM group can be explained by the significant epithelial atrophy we found as a result of diabetes. A greater amount of seminal fluid was produced as a result of caffeic acid treatment's reduction of epithelial cell histological alterations (Muhammad et al., 2021; Badescu et al., 2016). One of the main connections between diabetes and its problems is oxidative stress, which has been demonstrated to be brought on by hyperglycemia (Oyenihni et al., 2015). Free radicals are produced by hyperglycemia due to the autooxidation of glucose and the glycosylation of proteins (Al-Faris et al., 2010). Additionally, it seems that oxidative stress contributes to the development of nerve injury in both people and other experimental animal models of diabetes (Asmat et al., 2016; Knight et al., 2017; Kotian et al., 2019). Neurological dysfunctions brought on by oxidative stress include DNA damage, lipid peroxidation, reactive oxygen and nitrogen species production, and a reduction in cellular antioxidants (Khaki et al., 2010; Sidoryk et al., 2018). In order to ejaculate properly, the ductus ejaculatory closure resistance must be released, sympathetic neural input must be received, and seminal vesicles, ductus deferens, and vas deferens smooth muscle cells must contract in unison (Birowo et al., 2009; McKay et al., 2023). Therefore, it seems that oxidative stress brought on by diabetes has a negative effect on seminal vesicle innervation. The results of these studies indicate that oxidative stress brought on by diabetes is probably a secondary mechanism that results in seminal vesicle dysfunction. Caffeic acid treatment may reduce the parameters related to apoptosis and oxidative stress. It has been

demonstrated that caffeic acid possesses antioxidant qualities. More precisely, caffeic acid (3,4-dihydroxycinnamic acid) is a neuroprotective medication that inhibits both OH-dependent and OH-independent lipid peroxidation by inactivating hydroxyl radicals (OH) (Watanabe et al., 2003; Damasceno et al., 2017; Magnani et al., 2014).

Preserving the preganglionic neurons that innervate the seminal vesicles from oxidative damage brought on by hyperglycemia may be another strategy (Sun et al., 2021). Many organs are shielded by caffeic acid from oxidative stress and toxicity brought on by a variety of harmful assaults, including diabetes. It takes part in a number of vital biological functions, including immunity and membrane stability (Anuradha, 2009; Biaspo et al., 2017). Superoxide dismutase, glutathione peroxidase, glutathione reductase, catalase, and glutathione (GSH) have all been demonstrated to be secreted into the seminal fluid by the seminal vesicles, providing the spermatozoa with additional antioxidative protection (Giacco et al., 2010; Tramer et al., 1998).

As demonstrated by this work, the mechanism underlying caffeic acid's protective effect in seminal vesicular architecture and a secretory function could involve the increase of intracellular protein thiol and total thiol levels. Thus, the effects of severe oxidative stress may be mitigated by strengthening the organ's antioxidant defense. Nearly 50% to 80% of the ejaculated seminal plasma is secreted by seminal vesicles (Hussain et al., 2016). The importance of seminal fluid components for the growth and general health of the male offspring was recently shown by Bromfield et al., 2014. Additionally, Poiani (2006) emphasizes that the elements of mammals' seminal vesicular fluid are involved in: (1) sperm defensive function; (2) the stimulation of sperm ejection; (3) seminal blood clot formation; (4) suppression of neutrophils; (5) inhibition of the acrosome reaction; (6) regulation of immune activity throughout the female reproductive tract; (7) sperm transport by enhancement movements in the male and female genital tract; (8) antimicrobial activity; and (9) melting of the seminal fluid modifies sperm efficiency. The seminal vesicles in the muscle layer and the epithelium suffered significant histological impairment as a result of diabetes (Kumar et al., 2015; Lieberman et al., 2019). More specifically, we observed a highly condensed larger interior circular muscular layer and a slightly disorderly thin external longitudinal muscular layer (Asmat et al., 2016; Liu et al., 2017; Long et al., 2018). Furthermore, the internal muscular layer's muscle cells' cytoplasm seems to have shrunk in comparison to the control. Additionally, the smooth muscle cells had tiny vacuoles, and the muscle cells' nuclei seemed hyperchromatic, most likely due to the cells' strong reaction to the damage caused by diabetes (Ibrahim et al., 2015; Chao et al., 2009). The non-ciliated and basal cells, pseudostratified loft columnar epithelial cells make up the seminal vesicles' epithelium. Compared to the Control group, the DM group's epithelium displayed significant atrophy (Lal, 2016; Tripathi et al., 2014). The pseudostratified columnar epithelial cells appeared to have a decreasing height and declining cytoplasm, and the cell structure was totally chaotic (Moore et al., 2016; Murilo et al., 2019). Samples from every group showed the intricate papillary folds encircling the lumen. According to our research,

oxidative stress in the seminal vesicle may result in a decrease in the epithelium and a diminished testosterone action.

Conclusion

In this study, it was shown that the administration of Caffeic acid caused significant ameliorative effects against the reproductive and metabolic changes induced in diabetes in male Wistar rat models. Caffeic acid reduced hyperglycaemia, maintained body weight, restored antioxidant defence systems, enhanced testosterone production and protected the histoarchitecture of seminal vesicles, via its antioxidant activity and its role in insulin-associated glucose regulation. The present results indicate that caffeic acid might have therapeutic potential in reducing diabetes-induced reproductive dysfunction and oxidative tissue damage. When compared to the objective of the study, the results showed that caffeic acid significantly improved blood glucose level, decreased oxidative imbalance, bettered reproductive hormonal status and maintained the structure of S.V. of diabetic male Wistar rats. These histological and biochemical changes observed support the idea that antioxidant-based intervention could have an important role in decreasing reproductive complications of diabetes mellitus. The present study had a number of limitations, however, that must be recognized. Because of the relatively small number of observations per group, the results may not be statistically generalizable to the broader population and the results comparisons between groups may not be as robust. Furthermore, this study employed an experimental animal model and thus the results are preliminary and may not be directly applicable to physiological conditions in humans. The findings in this study may not be applicable across species due to differences in metabolism, endocrine control, or antioxidant responses. In addition, the study was mainly short-term and biochemical and histological effects were not studied and long-term reproductive performance, molecular signalling pathways, or dose dependence of caffeic acid were not investigated. Hence, more studies with larger sample size, longer treatment periods, molecular mechanistic studies and clinical trials are suggested to establish the therapeutic efficacy and safety of caffeic acid against diabetes induced reproductive dysfunction.

Recommendations

The results of the present study suggest a number of recommendations for future research and clinical or nutritional uses. Future studies should use larger sample sizes to provide increased statistical power, reproducibility, and generalizability of the results related to the protective effects of Caffeic acid against diabetes-induced reproductive dysfunction. Experimental diabetic models are recommended for comprehensive dose response study to ensure the optimum therapeutic dosage, duration and safety margin of caffeic acid. These investigations would provide data to determine if there are a dose-dependent protective effect and potential for toxicity. Further studies with human translatable experimental models are required to support the clinical relevance of the results. This may involve studies with higher mammalian models, translational biomedical models, and even

studies in well-designed clinical trials to examine the efficacy and safety of caffeic acid in human patients suffering from Diabetes mellitus.

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