ISSN: 2149-2018

Applied Medical Research, 2025
VOL 12, NO. 9, PAGE 1 – 9
DOI: doi.org/10.47363/AMR/2025(12)272

RESEARCH ARTICLE

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Anti-Diabetic and Anti-Inflammatory Effects of Caffeic Acid on the Seminal Vesicles and Penile Tissues of Diabetic Wistar Rats

Victor Okoliko Ukwenya[1], Oluwafemi Abidemi Adedotun[1,3]*, Olawale Olaleye Obembe[2] and Tiwalola Adebiyi[1]

[1]Department of Human Anatomy, School of Basic Medical Sciences, College of Health Sciences, Federal University of Technology, Akure, Nigeria

[2]Department of Physiology, College of Health Sciences, Osun State University, Osogbo, Nigeria

[3]Department of Human Anatomy, Elizade University, Ilara-Mokin, Ondo State, Nigeria

ABSTRACT

Background: Diabetes mellitus (DM) is a chronic metabolic disorder that constitutes a significant public health problem worldwide. Caffeic acid (CA) is reported to possess potent antioxidant and anti-inflammatory effects. This study aimed to investigate the effect of CA as a possible treatment of histopathological alterations of the penis and seminal vesicle in Fructose/Streptozotocin-induced diabetic Wistar rats.

Methods: Twenty (20) normoglycemic male adult Wistar rats weighing roughly 200g were used for this study. They were randomly divided into 4 groups: Control, which was given 0.1M citrate buffer daily, FRUCTOSE/STZ, which was given a single intraperitoneal injection of 50mg/Kg STZ following 2 weeks of 10% fructose ad libitum, FRUCTOSE/STZ+CA, which were also administered the same as the aforementioned group and treated with 50mg/Kg of Caffeic acid daily and CA group which was treated with 50mg/Kg of Caffeic acid also daily.

Results: The biochemical evaluation showed increased upregulation of NO and anti-inflammatory markers (Interleukin-4 and Interleukin-10) in the FRUCTOSE/STZ+CA compared to the FRUCTOSE/STZ. The penile tissues of FRUCTOSE/STZ diabetic rats showed sparse muscle layers and irregularly arranged thickened collagen fibres. The seminal vesicles of the diabetic control groups also featured disruption of the smooth muscle layer and enlarged lumina with scanty dispositions of fluid. Treatment with CA produced histological findings that were similar to the control group.

Conclusion: We conclude that CA ameliorated FRUCTOSE/STZ diabetic histopathology in rats' penis and seminal vesicles by boosting anti-inflammatory cytokines.

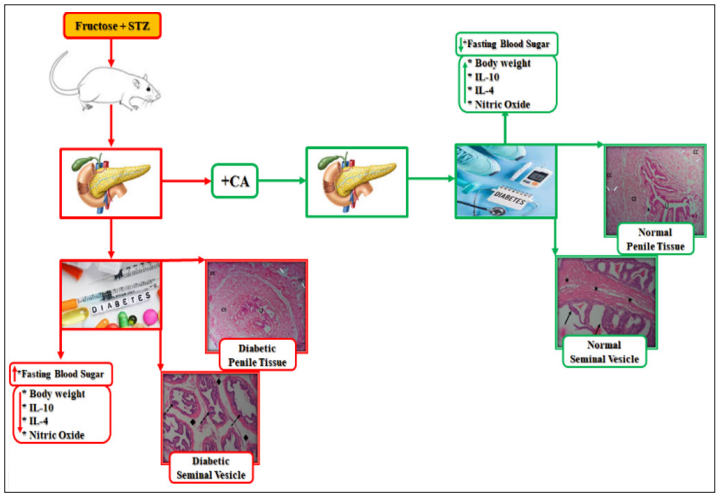
ARTICLE HISTORY

Received September 22, 2025
Accepted September 26, 2025
Published September 30, 2025

KEYWORDS

Diabetes,
Fructose-Streptozotocin,
Penis, Seminal Vesicle,
Caffeic Acid,
Histopathology

Graphical Abstract



Contact Oluwafemi Abidemi Adedotun, Department of Human Anatomy, Elizade University, Ilara-Mokin, Ondo State, Nigeria.

Introduction

Worldwide, diabetes mellitus (DM), a chronic metabolic disease, is a major health concern [1]. According to current estimates, around 4% of people globally have diabetes mellitus (DM), a number that is expected to rise to 5.4% by 2025 [2]. Up to 90% of diabetic individuals have sexual dysfunction, which includes infertility, reduced libido, and impotence [3]. It is believed that oxidative stress and redox imbalance are directly related to diabetic [4]. Male sexual dysfunctions are often classified according to these categories: male erectile dysfunction (ED), ejaculatory dysfunction, decreased libido, orgasmic, and refractory period dysfunction. Men with diabetes mellitus are more likely to have two of these disorders: ejaculatory dysfunction and erectile dysfunction [5]. Since sexual health is a significant component of total well-being for men who engage in sexual activity, ED has been seen as a public health concern [1]. Diabetic males have a threefold increase in the risk of ED when compared to non-diabetic men [6]. Testicular androgens regulate accessory sex organs like the seminal vesicle, and any alteration in the levels of androgens in the blood would be reflected in the weight and size of these organs [7]. There have been reports of decreased androgen receptor content, absorption, and retention in accessory sex glands in adult streptozotocin-diabetic rats [8].

Numerous plant items, including fruits, vegetables, coffee, tea, wine, cereals, and Chinese medicinal herbs, contain caffeineic acid (3, 4-dihydroxycinnamic acid) (CA) [9]. Caffeic acid has exhibited pharmacological antimutagenic activities, antioxidant, antivirus, anticancer, anti-inflammatory properties, and antidiabetic effect [1,10]. This study investigated the therapeutic role of caffeic acid on the penile tissue and seminal vesicles of animals with experimentally induced hyperglycaemia.

Materials and Methods

Animal Care and Management

Twenty (20) mature male Wistar rats weighing 236 ± 38g and normoglycemic (64 ± 15 mg/dl) were acquired from the animal house of the Department of Anatomy at Ekiti State University in Ekiti, Nigeria. They were taken to the anatomy department's Animal House at the Federal University of Technology Akure in Ondo State, Nigeria, where they were kept in polycarbonate cages with a 12-hour light-dark cycle and a regulated room temperature of around 30oC. For two weeks, all of the rats were allowed to freely consume tap water and standard rat chow as they acclimated.

Experimental Design

Two (2) random sets of five (5) rats each were chosen for the Control group and the Caffeic acid (CA) group, while the remaining ten (10) rats were given 10% fructose solution ad libitum for two weeks, followed by a single intraperitoneal injection of 50 mg/kg of streptozotocin (STZ). The 10 rats were subsequently divided into two groups of five: Fructose-Streptozotocin (FRUC-STZ) and Fructose-Streptozotocin + Caffeic Acid (FRUC-STZ + CA).

All animal-related procedures in this study were approved by the Departmental Committee on the use and care of animals and adhered to the guidelines for animal research as recommended by the Helsinki Declaration and the guidelines in the care and use of animals [11].

The rats were randomly divided into the following experimental groups:

- Normal control = 2ml citrate buffer
- Diabetic control (FRUC-STZ) = 2 weeks 10% fructose solution ad libitum + 50mg/kg body weight STZ
- FRUC-STZ + Caffeic acid (FRUC-STZ + CA) = 2 weeks 10% fructose solution ad libitum + 50mg/kg body weight STZ + 50mg/kg body weight CA
- Caffeic Acid only = 50mg/kg body weight CA

Caffeic acid (50 mg/kg) was dissolved in distilled water and administered orally. The Control group was also given citrate buffer orally.

Tissue Harvesting and Processing

Following euthanasia using sodium pentobarbital, injected intraperitoneally, the animals were dissected by abdominopelvic incision at the conclusion of the experiment, and the seminal vesicles and penile tissues were removed and prepared for histological analysis in accordance with earlier protocol [12]. Afterwards the slides were mounted in DPX and stained with hematoxylin and eosin (H&E) and Masson Trichrome stains, photomicrographs were captured on an OMAX microscope (USA) at a magnification of X100.

Serum Preparation from Blood Samples

Using a cardiac puncture procedure, blood samples were collected into sterile, plain bottles and left to clot for two (2) hours at room temperature. The sera were obtained by centrifugation in a centrifuge set at 2500 rpm for 20 minutes. Afterwards, using micropipettes to aspirate the sera from various samples into the corresponding sterile plastic sample bottles with labels, the samples were promptly placed in the refrigerator for the biochemical analysis.

Statistical Analysis

One-way ANOVA and Tukey's comparison test were used for statistical analysis of the collected data. The mean ± SEM was used to express the data. Unless otherwise indicated, the significance threshold was set at $p < 0.05$. GraphPad Prism 8 Windows (GraphPad Software, San Diego, California, USA) was used to analyze the data.

Results

Effect of Caffeic Acid on the Total Body Weight of Fructose-STZ Induced Experimental Rats

Data for total body weight are expressed in Table 1. The Fructose-STZ diabetic group (FRUC-STZ) shows the highest decline when comparing the initial weight and final weight. The treatment groups and control group show minimal difference between the initial weight and final weight.

Table 1: Table Showing the Initial and Final Body Weight Changes in the Experimental Animals after a Period of 14 Days. Values Represent Mean ± SEM; n = 5.

	CONTROL	FRUC-STZ	FRUC-STZ+CA	CA
Initial weight (g)	212.3±11.1	206.8±9.8	213.6±9.3	228.8±12.7
Final weight (g)	202.3±9.1	167.3±8.2*	210.2±11.7	209.6±10.0
Weight decrease (%)	4.71	18.71*	1.59	8.39

* Denotes significant decrease at $p < 0.05$ to the Control, FRUC-STZ+CA, and CA groups.

Effect of Caffeic Acid on the Total Blood Sugar of Fructose-STZ Induced Experimental Rats

Table 2 contains data for the total blood sugar of the animals. The FRUC-STZ group showed a high average final glucose level in the diabetic range (234.0mg/dL) while the FRUC-STZ+CA group showed final blood glucose levels closer to the control group and CA group.

Table 2: Table Showing the Initial and Final Blood Glucose Changes in the Experimental Animals after a Period of 14 Days. Values Represent Mean ± SEM; n = 5

	CONTROL	FRUC-STZ	FRUC-STZ+CA	CA
Initial glucose (mg/dL)	47.5±2.9	306.3±16.5 *	340.3±31.0 *	57.2±2.0
Final glucose (mg/dL)	76.8±3.6 #	234.0±32.6	116.3±38.6 #	72.6±3.7 #
Blood glucose Change (%)	61.7	23.6	65.8	26.9

* Denotes significant increase at $p < 0.01$ relative to the Control group.

# Denotes significant decrease at $p < 0.01$ relative to the FRUC-STZ group.

Interleukin-10 (IL-10) and Interleukin-4 (IL-4) Expressions on the Effect of Caffeic Acid on Fructose-STZ Induced Experimental Rats

In this study, the FRUC-STZ group's IL-10 and IL-4 levels was significantly lower ($p<0.05$) than that of the Control group (Fig. 1). Additionally, compared to the FRUC-STZ group, the IL-10 and IL-4 levels of the FRUC-STZ+CA and CA only groups increased significantly ($p<0.05$) (Fig. 1). Although, when compared to the Control, the FRUC-STZ+CA and CA only groups' IL-10 levels share no significant difference (Figure 1).

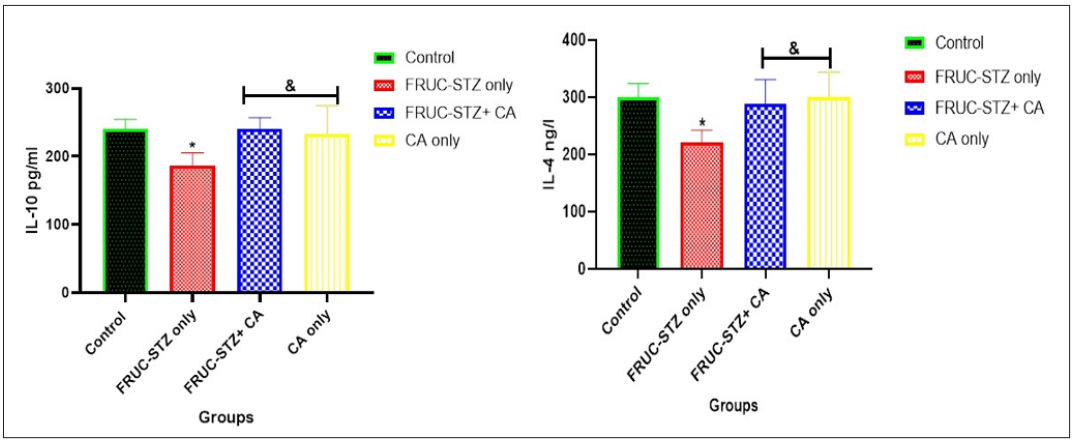


Figure 1: Interleukin-10 and Interleukin-4 Expressions on Experimental Animals

* Statistically significant different from control at $p < 0.05$; & statistically significant different from FRUC-STZ group at $p < 0.05$

Effect of Caffeic Acid on the Nitric Oxide Levels of Fructose-STZ Induced Experimental Rats

In this study, the FRUC-STZ group's Nitric oxide (NO) level was significantly lower ($p<0.05$) than that of the Control group (Figure 2). In contrast to the FRUC-STZ group, the FRUC-STZ+CA and CA only groups' NO levels increased significantly ($p<0.05$) (Figure 2). When compared to the Control, the FRUC-STZ+CA and CA only groups' NO levels were not significantly different (Figure 2).

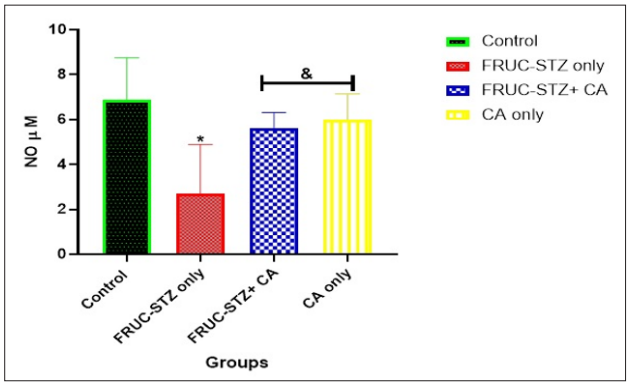


Figure 2: Nitric Oxide (NO) Level in Experimental Animals

**** Statistically significant different from control at p < 0.05; & Statistically significant different from FRUC-STZ group at p < 0.05***

Histoarchitecture of Penile Tissues

Haematoxylin and Eosin Stained Penile Histomorphology

The penile tissues stained with H & E in this study showed the three erectile tissues which include the ventrally located corpus spongiosum which covered the urethra, and the paired corporal cavernosa with cavernous sinuses in the control group (Figure 3A). In the group administered with FRUC-STZ only, the penile tissues showed a stricture in the shape and texture of the lumen with visible scar tissues (Figure 3B). Furthermore, a distortion and disruption in the arrangement pattern of the cavernous sinuses of the corporal cavernosa together with a less spongy-like texture of the corpus spongiosum were also observed in the group administered with only FRUC-STZ (Figure 3B). The penile tissues of the group administered with FRUC-STZ followed by treatment with Caffeic acid showed a clear urethral lumen with spongy-like corpus spongiosum and a less disrupted and closely arranged cavernous sinuses of the corporal cavernosa (Figure 3C). The group administered with Caffeic acid only showed penile tissues similar to that of the control with wide urethra a clear lumen and normal histoarchitecture of the erectile tissues (Figure 3D).

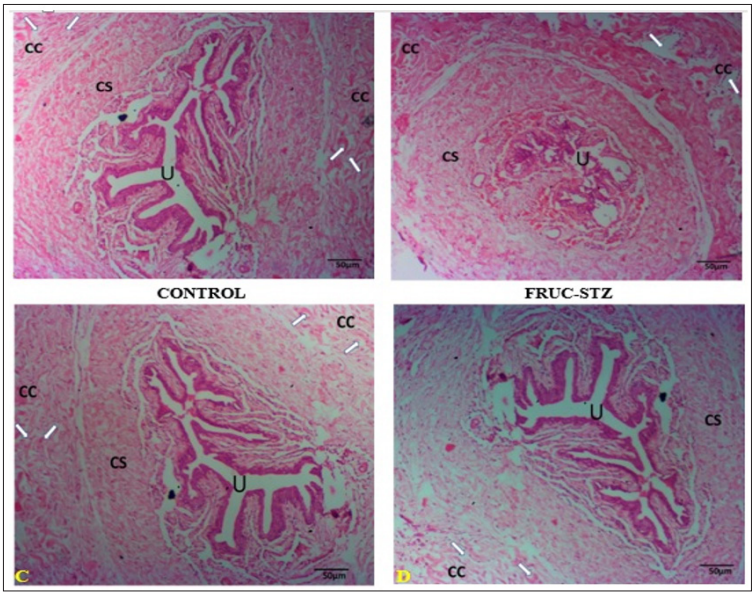


Figure 3: Representative Penile Tissue photomicrograph of the Control group and Experimental groups.

U (Urethra); CC (Corporal Cavernosa); CS (Corpus spongiosum); White Arrows (Cavernous sinuses). Stains: H&E. Magnification: X 100

Masson Trichome Stained Penile Histomorphology

The control group's penile tissues stained with Masson trichome showed the corpus cavernosa's trabeculae, which are made up of collagen fibers and smooth muscle fibers in a regular network arrangement (Figure 4). In the group administered with FRUC-STZ only, the penile tissues showed sparse muscle layers and increased diameter and thickness of collagen fibers with irregular arrangement patterns (Figure 4). The group administered with FRUC-STZ and Caffeic acid showed improvement in the penile tissues presented by decreased diameters and decreased thickness of collagen fibers with regular arrangement pattern. In the group administered Caffeic acid only, the collagen fibers' trabeculae likewise showed a reduction in thickness and a regular network resembling the control group's (Figure 4).

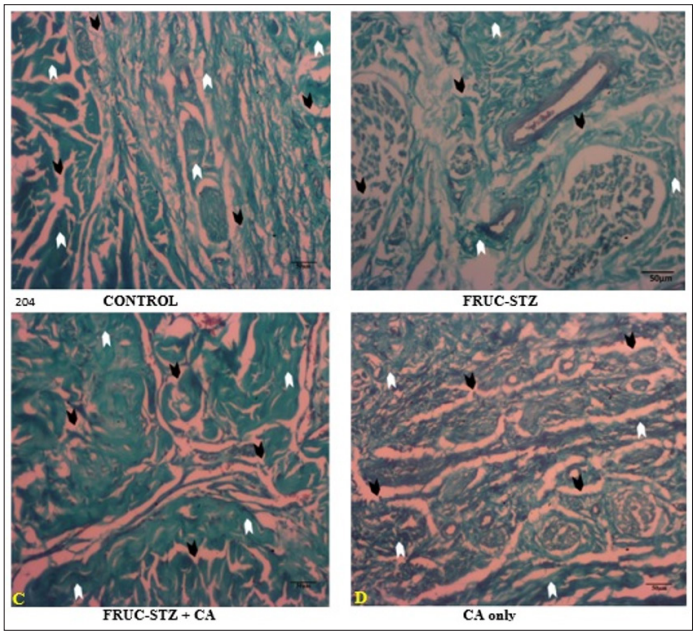


Figure 4: Representative Penile Tissue Photomicrograph of the Control and Experimental Groups.

The Black downward arrows indicate the unstained area-sinusoids spaces (the muscle layer) The White upward arrows indicate blue staining signifying the collagen fibers and their extracellular matrixes. Stain: Masson trichome. Magnification: X100

Histoarchitecture of Seminal Vesicles

The Control group's seminal vesicles had honeycombed saccules with thin, highly branching mucosal folds and a pseudostratified columnar epithelium lining (Figure 5). In the FRUC-STZ group, there is disruption of the smooth muscles at the saccular dilation of the gland compared to that of the control group (Figure 5). The lumina is enlarged with scanty dispositions of fluid. The connective tissue stroma is disrupted with attenuation of the smooth muscle fibres. Tissues from the therapeutic FRUC-STZ+ CA group showed well-defined structures that are comparable with the control group (Figure 5). The histoarchitecture of the CA only group alo showed well delineated honeycombed saccules with mucosa lined by pseudostratified columnar epithelium (Figure 5).

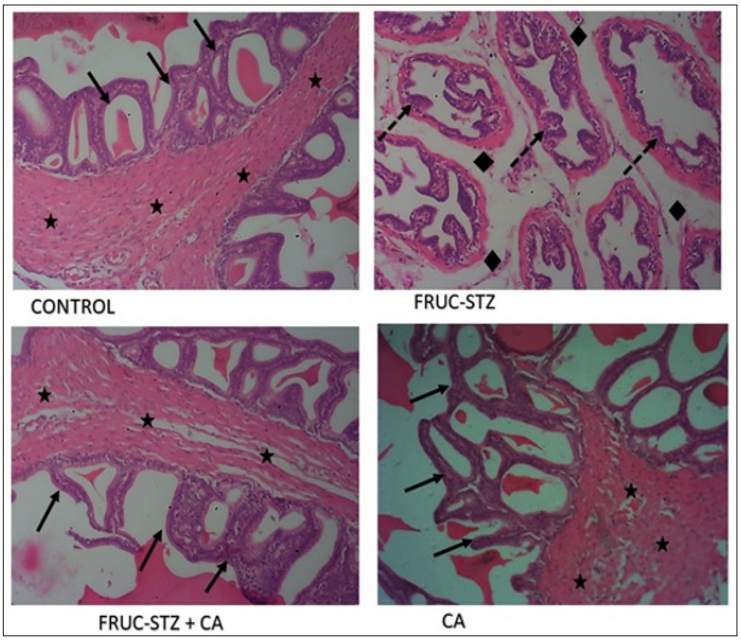


Figure 5: Representative Seminal Vesicle Photomicrograph of the Control and Experimental Groups.

Stars represent smooth muscles; arrows indicate well-defined epithelial tissues; Rhomboids indicate disrupted smooth muscle layer and broken arrows indicate disrupted epithelial cells. Stain: H&E. Magnification: X 100.

Discussion

Numerous environmental agents, such as pharmaceuticals, cosmetics, agrochemicals, environmental pollutants, and infections including bacteria, viruses, and parasites, can cause harmful impacts to human reproductive functions [13]. These substances' detrimental effects on the reproductive systems are considered to be the main factor contributing to the global rise in infertility [14]. An overlooked yet prevalent complication of diabetes is sexual dysfunction. Erectile dysfunction (ED), libido abnormalities, and ejaculatory issues are examples of male sexual dysfunction in diabetic individuals [15]. According to Lue, erectile dysfunction (ED) is a prevalent and complicated illness that has a major negative influence on quality of life and is acknowledged as a serious public health issue [16]. Caffeic acid however has been proven to regulate lipid peroxidation and inhibit lipo-oxygenase activities [17]. It also possesses anti-inflammatory, immunomodulatory, antiproliferative, and antioxidant properties [17]. This study however presents the influence of Caffeic acid on Fructose-streptozotocin induced diabetes on the penis of adult male Wistar rats.

Changes in body and organ weight are highly sensitive markers of changes in animals induced by chemicals [18]. The result from this study showed a significant drop in the initial and final weight of the FRUC-STZ only animals when likened to that of the Control and CA treated groups. This result is in agreement with a study by Ukwenya et al. [12]. There has been evidence linking STZ-induced diabetes to overall weight reduction [18]. The FRUC-STZ+CA group showed final blood glucose levels closer to the control group and CA only group is due to the heightening of insulin levels. Caffeic acid's ability to reduce the glucose level to normal following STZ induction is consistent with a previous study by Fuliang et al. [19]. The rise in insulin by Caffeic acid may have come from enhanced insulin production from pancreas and/or regeneration of pancreatic β-cells, as has been postulated in earlier research [20].

Among all anti-inflammatory cytokines, interleukins 10 and 4 (IL-10 and IL-4) have strong anti-inflammatory effects and inhibit activated macrophages' production of inflammatory cytokines such TNF-α, IL-6, and IL-1 [21]. The result from this study showed a significant decrease in the IL-10 and IL-4 level of the FRUC-STZ group when compared with the Control. A prior work by Sandberg et al. documented the functions of pro-inflammatory cytokines in islet inflammation and β-cell damage, as seen by a reduction in anti-inflammatory cytokine levels in rats with STZ-induced hyperglycemia, is consistent with our conclusion [22]. However, a significant increase was observed in the IL-10 and IL-4 levels of the FRUC-STZ+CA and CA only group when compared with the FRUC-STZ only group. This can be said to be as a result of the anti-inflammatory properties of Caffeic acid as documented by Michaluart et al. and Aksoy et al [23,24].

The primary biomolecule involved in penile erection is nitric oxide (NO) [25]. The NO level of the FRUC-STZ group was significantly lower than that of the Control group, according to this study's findings. NO is found in many parts of the human body and is thought to play a key role in both physiological and pathological processes, including the mechanism behind penile erection [26]. However, a significant increase in the NO levels of the FRUC-STZ+CA and CA only groups were observed when compared with the FRUC-STZ only group. Since it makes up for the drawbacks of phosphodiesterase type 5 (PDE5) inhibition, augmentation of NO levels and

associated nitric oxide synthase enzyme activity is thought to be an alternate treatment target for ED [27]. Numerous scientific studies have looked at how natural compounds affect ED, and Caffeic acid has showed a lot of promise [28,29].

In diabetic ED models, fibrosis is a significant degenerative process that is associated by neuropathy, endothelial degradation, and decreased smooth muscle [30]. In the flaccid condition, collagen fibers are often organized in an undulating pattern and are made up of aggregations of tropocollagen molecules. The penis may undergo mechanical changes that result in decreased elasticity and compliance due to changes in collagen types or elastic fibers [31]. From this study, the penile tissues stained with Masson trichrome showed sparse muscle layers and increased diameter and thickness of collagen fibers with irregular arrangement patterns in the FRUC-STZ only group, which is in contrast to the control that displayed the corpora cavernosa's trabeculae made up of collagen fibers and smooth muscle fibers, arranged in a regular network. This outcome is consistent with a prior study that found that the STZ rat penis had a large increase in connective tissue deposition and a significant decrease in trabecular smooth muscle content [32]. The loss of corporal smooth muscle cells due to neuropraxia-induced apoptosis may be the cause of the increased fibers in penile tissue, which may result in fibrosis of the penile corpora cavernosa [33]. Additionally, after receiving STZ, diabetic rats' smooth muscle and endothelial cell content significantly decreased, according to Ahn et al [34]. However, the animals administered with FRUC-STZ and caffeic acid showed improvement in the penile tissues presented by decreased diameters and decreased thickness of collagen fibers with regular arrangement patterns in the Masson trichome stained penile tissues and a clear urethral lumen with spongy-like corpus spongiosum and a less disrupted and closely arranged cavernous sinuses of the corporal cavernosa in the H & E-stained penile tissues. These ameliorative effects by Caffeic acid may be attributed to its various cytoprotective effects [35]. Additionally, according to earlier studies, Caffeic acid esters show comparable anti-diabetic effects [10].

Conclusion

In conclusion, by elevating the levels of strong anti-inflammatory cytokines (IL-10 and IL-4) and nitric oxide in diabetic rats, Caffeic acid has been shown to have anti-diabetes benefits. Additionally, Caffeic acid showed great potential in mitigating the histopathological consequences of diabetes on penile tissues and seminal vesicles. These findings suggest that caffeic acid may be used as a supplement to treat erectile dysfunction and infertility related to diabetes.

Ethical Approval

The authors declare that all animal-related procedures in this study were approved by the Departmental Committee on the use and care of animals and adhered to the guidelines for animal research as recommended by the Helsinki Declaration and the guidelines in the care and use of animals (National Research Council, 2011). It was approved by the Health Research and Ethics Committee of the Federal University of Technology, Akure (CHS/FUA/2023/048).

Consent for Publication

Not applicable

Competing Interest

The authors report no conflict of interest.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' Contributions

- Victor Okoliko Ukwenya: Conceptualization, Methodology, Validation, Writing -review and editing, Investigation
- Oluwafemi Abidemi Adedotun: Methodology, Project administration, Supervision, Investigation, Graphical Abstract
- Olawale Olaleye Obembe: Formal analysis, Investigation, Proof-reading and Formatting
- Tiwalola Adebiyi: Writing-original draft, Proof-reading and Formatting

Acknowledgments

Special appreciation goes to the Department of Anatomy at Ekiti State University, Ekiti, Nigeria for ease of procurement of the Wistar Rats used for this experiment.

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