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Original Research Article

Protective effects of combined extracts of *Allium cepa, Allium sativum and Citrullus lanatus* on the testis and semen parameters in Streptozotocin-induced diabetes in adult male wistar rats

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Abstract

Purpose: *To investigate the protective and androgenic effects of combined extracts of Allium cepa (onion), Allium sativum (garlic), and Citrullus lanatus (watermelon) on testicular histology and semen parameters in streptozotocin-induced diabetic male Wistar rats.*

Methods: *Twenty male Wistar rats were divided into five groups: control given distilled water (10 mL/kg), diabetic control, Viagra-treated (10 mL/kg), low-dose combined extracts (250 mg/kg), and high-dose combined extracts (500 mg/kg) orally administered through a gavage. Diabetes was induced with a single intraperitoneal injection of streptozotocin at 45 mg/kg. Following confirmation of hyperglycemia, animals received once-daily oral administration of the combined plant extracts for 21 days at doses of 250 and 500 mL/kg or Viagra (10 mL/kg). The experiment was terminated on day 22. Semen analysis assessed pH, sperm motility, viability, and count. Testicular tissues were examined histologically. Data were analyzed using one-way ANOVA with Tukey's post-hoc test, considering $p < 0.05$ as statistically significant.*

Results: *Diabetic control rats exhibited significant reductions in sperm motility, viability, and sperm count ($p < 0.05$), and histological abnormalities such as thickened seminiferous tubules and vacuolations in comparison with control. Treatment with low and high doses of the combined extracts significantly improved semen parameters, approaching control levels ($p < 0.05$), with histology revealing near-normal testicular architecture. The effects of the combined extracts show improvements in sperm quality approaching the levels of the control and Viagra, likely via their antioxidant and androgenic mechanisms.*

Conclusion: *The combined extracts of Allium cepa, Allium sativum, and Citrullus lanatus effectively mitigate diabetes-induced reproductive impairments in male rats, supporting their potential as natural, safe interventions for improving male reproductive health in diabetic conditions.*

Keywords: *Allium cepa, Allium sativum, Citrullus lanatus, Male reproductive health, Semen parameters, Streptozotocin-induced diabetes, Testicular histology*

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INTRODUCTION

Reproductive health is a vital aspect of male vitality and fertility, heavily dependent on the proper functioning of the testes and the integrity of semen parameters such as sperm count, motility, morphology, and hormonal balance [1]. These parameters are crucial indicators of male reproductive capacity and overall reproductive success [2]. Diabetes mellitus, a chronic metabolic disorder characterized by hyperglycemia, has been increasingly linked to reproductive dysfunction in males [3,4]. The condition induces oxidative stress, hormonal imbalances, vascular damage, and tissue atrophy, which collectively impair spermatogenesis and testosterone production [5]. Studies have shown that diabetic males often exhibit reduced sperm quality, decreased libido, and testicular degeneration, ultimately compromising fertility [6].

Streptozotocin (STZ) is a chemical agent widely used in experimental models to induce diabetes in animals by selectively destroying pancreatic beta cells [7]. STZ-induced diabetic models mimic human type 1 diabetes and are invaluable for studying the pathophysiology of diabetes-related complications, including reproductive impairments [8]. Current therapeutic options for diabetic reproductive dysfunction are limited and often involve pharmacological agents that may cause adverse effects [9]. As a result, there is an increasing interest in exploring natural remedies with antioxidant, anti-inflammatory, and aphrodisiac properties to mitigate these complications [10].

Traditional medicine suggests that plants such as *Allium cepa* (onion), *Allium sativum* (garlic) of the *Amaryllidaceae* family and *Citrullus lanatus* (watermelon) of the *Cucurbitaceae* family possess potent bioactive compounds such as polyphenols and flavonoids [11–13]. These plants are known for their antioxidant properties [14–16], which could potentially counteract oxidative stress-induced damage in reproductive tissues [17]. Moreover, they are believed to have aphrodisiac effects and enhance reproductive parameters [18–20].

However, scientific validation of the combined effects of these plants, especially in the context of diabetic reproductive dysfunction, remains limited. This study aims to evaluate the androgenic and protective effects of combined extracts of *Allium cepa, Allium sativum,* and *Citrullus lanatus* on the testes and semen parameters in adult male Wistar rats with streptozotocin-induced diabetes. The findings could provide insights into natural, safe, and effective interventions to improve reproductive health in diabetic males.

EXPERIMENTAL

Animals

Twenty (20) male adult Wistar rats with an average weight range of 200–280 g were used for this experiment. The rats were obtained from the medical college animal house, University of Calabar, Calabar. They were housed in well-ventilated plastic and wire-gauze netted cages in the university animal house. The animals had a complete light cycle of 12 hours and considerable darkness of 12 hours. The rats were fed daily with grower's mash and given water *ad libitum*. Twenty (20) male Wistar rats were kept for acclimatization for a period of two weeks.

Ethical approval

Approval was obtained from the Faculty Animal Research Ethics Committee (FAREC-FBMS), Faculty of Basic Medical Sciences, University of Calabar, Calabar (no. 124ANA3982). All procedures were conducted in strict accordance with recognized international guidelines for the care and use of laboratory animals, specifically adhering to the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals.

Plant collection and identification

Watermelon, white onion and garlic were bought at Watt Market, Calabar, Cross River State, Nigeria (4.957642° N, 8.321507° E; 4°57′28″ N, 8°19′17″ E). They were identified by a taxonomist of the Department of Plant and Ecological Science, University of Calabar, Calabar and voucher numbers for *Allium cepa* (Herb/bot/ucc/0302), *Allium sativum (*Herb/Bot/ucc/0303), and *Citrullus lanatus* (Herb/Bot/ucc/0626) were given.

Preparation for juice extraction

Fresh watermelon, fresh bulbs of white onion and cloves of garlic, which were brought to the laboratory, were thoroughly sorted into different containers and rinsed/washed with running tap water to remove traces of dust and unwanted materials. Equal quantities of the different plants were weighed, cut into smaller pieces with a knife and blended using an electric blender (Vitamix 5200, USA).

Juice extraction procedure

The watermelon sample was accurately weighed to get an equal quantity using a weighing balance model (AE Adam); garlic and white onion were weighed (500 g) each.

The resulting juice was filtered to remove particulate matter and impurities, then concentrated using a rotary evaporator to reduce excess moisture and enrich its bioactive constituents. Finally, the concentrate was preserved at −20 °C until required for analysis.

Induction of diabetes

Diabetes was induced using 45 mg/kg of streptozotocin (STZ) from Sigma-Aldrich (African Biosciences Ltd, Ibadan, Oyo State, Nigeria). Three days after induction, diabetes was confirmed using peripheral whole blood obtained via lateral tail vein puncture and a FineTest Auto-Coding Premium Glucometer, and animals with blood glucose exceeding 11.1 to 13.9 mmol/L were considered diabetic. Administration of the combined extracts began and distinctive marks were made on each rat to differentiate the individual rats and groups.

Group allocation

The animals were divided into five groups (n = 5): normal control (distilled water 10 mL/kg), diabetic control, Viagra (sildenafil citrate) from Pfizer, United States (headquartered in New York, USA) 10 mL/kg, combined juice extracts at 250 mg/kg (low dose) and 500 mg/kg (high dose). The juice extract was administered orally using a gavage for a period of 21 days, once daily.

Sample collection

At the end of 21 days of administration, animals were sacrificed a day after the last dose, after being euthanized with chloroform. The testes were carefully harvested from the various experimental groups and were fixed in 10% neutral buffered formalin.

The epididymis was dissected out and placed in physiological saline in a ratio of 1:10 weight (g) to volume (mL), and it was then macerated using a surgical blade to release the sperm cells.

Determination of seminal parameters

The following sperm parameters were analyzed as follows:

Semen pH

The epididymis was punctured with a sterile blade, and the sperm smear on the blade was rubbed on a narrow-range pH paper ranging from 6.0 to 8.0. The color changes were matched with those of the pH chart and the values were recorded accordingly.

Sperm motility

Two drops of sperm suspension were put on a clean and labeled microscope slide and covered with a coverslip. This was mounted on a light microscope and the number of motile cells was divided by the total number of sperm cells counted and was expressed as a percentage.

Sperm viability (%)

The method employed was the eosin-nigrosin staining technique. The semen suspension mixture was added to an equal volume of the stain and smeared on glass slides.

After staining, dead sperm cells adopted a pink color, while live sperm cells displayed lighter coloration. The live sperm cells counted were divided by the total sperm cell number and multiplied by 100.

Sperm count ($\times 10^6$/mL)

An improved Neubauer hemocytometer was used for the sperm count. A capillary tube was used to pipette the sperm suspension into the chamber of the Neubauer hemocytometer. The Neubauer hemocytometer was placed on a light microscope and the counting was estimated by multiplying the number of cells counted by the dilution factor and by the hemocytometer volume.

Histological processing

The testes were harvested, fixed in 10% formalin, and processed for histological examination using the hematoxylin and eosin (H&E) staining technique. The sections were examined using a light microscope, and the changes in the testicular tissue were evaluated.

Statistical analysis

The seminal parameters were measured, and the data were analyzed using a one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test; data were presented as mean ± standard error of the mean (SEM). $P < 0.05$ was considered statistically significant.

RESULTS

Histological observations of the testis

Histological examination revealed that STZ-induced diabetic rats showed notable testicular tissue damage, including degeneration of seminiferous tubules, germ cell loss, and disorganized cellular architecture. In contrast, groups treated with the combined juice extract of *Allium cepa, Allium sativum,* and *Citrullus lanatus* displayed varying degrees of histological improvement, characterized by preservation of seminiferous tubule structure, increased germ cell proliferation, and reduced tissue degeneration (Figure 1).

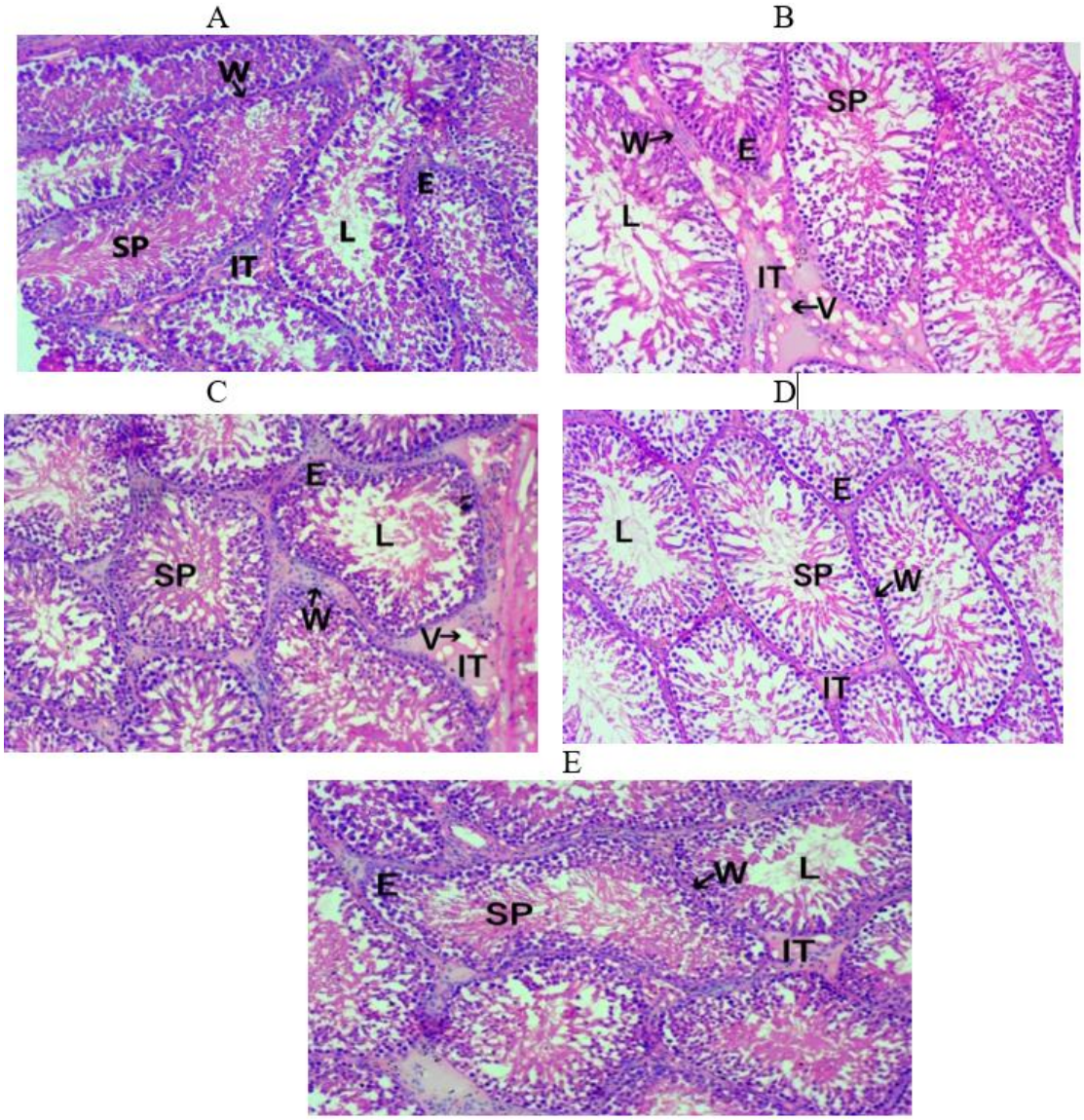


Figure 1: Photomicrograph of histological section of the testis of (A) control group with central lumen filled with mature spermatozoa and many cell layers are found in the germinal epithelium; (B) diabetic control containing thickened walls of seminiferous tubules separated by broadened interstitial space that is filled with numerous vacuoles. The central lumen is filled with few mature spermatozoa, and few cell layers are found in the germinal epithelium; (C) Viagra group, showing minimally thickened walls of seminiferous tubules separated by broadened interstitial space that is filled with numerous vacuoles. The central lumen is filled with few mature spermatozoa. Few cell layers are found in the germinal epithelium; (D) low-dose combined juice extract group, showing normal walls of seminiferous tubules. The interstitial space has normal morphology. The central lumen is filled with numerous mature spermatozoa. Many cell layers are found in the germinal epithelium. (E) High-dose combined juice extract group, showing mild thickening of the wall of seminiferous tubules. The interstitial space has normal morphology. The central lumen is filled with numerous mature spermatozoa. Many cell layers are found in the germinal epithelium. **Key:** Walls of seminiferous tubules (W), interstitial space (IT), central lumen (L), spermatozoa (SP), germinal epithelium (E). H&E ×100 magnification

Seminal parameter analysis

pH

No significant changes in the pH of the semen were observed among the different groups ($p > 0.05$).

Percentage sperm motility

There was a significant decrease in sperm motility in diabetic control, low-dose, and high-dose combined juice extract groups compared to the normal control. However, both the low- and high-dose combined juice extract groups showed a significant increase in sperm motility compared to diabetic control, with a notable decrease when compared to the Viagra-treated group ($p < 0.05$).

Percentage non-motile sperm

The percentage of non-motile sperm was significantly higher in diabetic control compared to the control. Treatment with the combined plant extracts at low and high doses resulted in a significant reduction in non-motile sperm compared to diabetic control, although it was significantly higher when compared to the Viagra group ($p < 0.05$).

Percentage viable sperm

Diabetic control group exhibited a significant decrease in sperm viability compared to the control group. Treatment with the combined juice extracts led to a significant increase in viable sperm percentage relative to diabetic control, although viability remained significantly lower than in the Viagra group ($p < 0.05$).

Sperm count: There was a significant reduction in sperm count in diabetic control and low-dose combined juice extract groups compared to the control. However, both the low- and high-dose groups showed a significant increase in sperm count compared to diabetic control, approaching normal levels ($p < 0.05$).

DISCUSSION

This study investigated the potential protective and androgenic effects of combined extracts of *Allium cepa* (white onion), *Allium sativum* (garlic), and *Citrullus lanatus* (watermelon) on testicular histology and semen parameters in streptozotocin (STZ)-induced diabetic male Wistar rats. Diabetes mellitus is well-documented to impair male reproductive function through mechanisms such as oxidative stress, hormonal imbalance, and testicular tissue degeneration [3]. The findings of this study provide insight into the extent of reproductive impairment caused by diabetes and the potential ameliorative effects of the combined plant extracts. Diabetic rats exhibited significant reductions in sperm motility, viability, and sperm count, alongside histological evidence of testicular degeneration characterized by thickened seminiferous tubule walls, vacuolations, and decreased germinal cell layers. These findings align with previous studies indicating that hyperglycemia-induced oxidative stress damages spermatogenic cells, impairs Leydig cell function, and leads to decreased testosterone production, ultimately compromising spermatogenesis [5]. The decrease in sperm motility and viability is indicative of impaired sperm function, which is crucial for fertility [21]. Histologically, diabetic testes showed thickened seminiferous tubule walls and vacuolations, suggestive of tissue atrophy and degeneration. These morphological changes further corroborate the functional impairments observed in semen analysis. Treatment with low and high doses of the combined *Allium cepa, Allium sativum,* and *Citrullus lanatus* juice extracts resulted in significant improvements in semen parameters compared to diabetic control rats. Notably, both doses significantly increased sperm motility, approaching levels comparable to the control and Viagra-treated groups. This suggests enhanced sperm function, potentially due to antioxidant properties mitigating oxidative stress.

Table 1: Semen pH, Sperm percentage motility and viability changes and sperm count changes in STZ-induced rats treated with the combined juice extracts

Group	pH	Motile (%)	Non-motile (%)	Viable (%)	Non-viable (%)	Count ($\times 10^6$/mL)
Control	6.97±0.09	26.94±1.62	4.35±2.17	30.33±0.85	2.75±1.57	67.87±1.95
Diabetic control	6.95±0.05	6.66±2.43*	11.96±3.26*	11.45±1.59*	8.84±2.95*	21.70±8.30*
Viagra	6.81±0.01	29.88±0.02	8.68±0.02*	25.14±0.003*	2.95±0.006	48.41±0.01
Low dose	7.15±0.15	16.39±0.81*a,b	35.88±1.09*a,b	13.20±0.80*a,b	39.78±1.47*a,b	20.70±0.90*a
High dose	6.80±0.20	20.13±0.0003*a,b	39.14±0.004*a	19.88±0.80*a,b	45.67±1.47*a,b	36.50±28.40*a

Values are expressed as mean ± SEM, n = 5; means with asterisks (*) are significantly different from Control; means with 'a' are significantly different from Diabetic Control; b = significantly different from Viagra (250 mg/kg) at $p < 0.05$

The juice extracts improved sperm viability and count significantly compared to diabetic rats, indicating a restorative effect on spermatogenesis. The histological examination supports this, as the seminiferous tubules in treated groups showed fewer degenerative changes and more normal germinal epithelium. The testicular tissues in the juice extract-treated groups displayed near-normal architecture with normal seminiferous tubule walls, reduced vacuolations, and abundant mature spermatozoa in the lumen, corroborating the functional sperm parameters. The Viagra group maintained higher sperm motility and viability compared to diabetic controls, consistent with its known vasodilatory and erectile function enhancement effects [9]. However, the plant extracts demonstrated comparable improvements in semen parameters, indicating their potential as natural alternatives or adjuncts in managing diabetes-induced reproductive dysfunction. The bioactive compounds in *Allium cepa, Allium sativum,* and *Citrullus lanatus* are known for their antioxidant, anti-inflammatory, and androgenic properties [20]. These plants contain flavonoids, saponins, and other phytochemicals such as organosulfur compounds and carotenoids capable of scavenging free radicals, reducing oxidative stress, and protecting Leydig and germ cells from damage [18]. Their combined use may synergistically enhance testicular function, promote spermatogenesis, and restore hormonal balance. Furthermore, *Citrullus lanatus* (watermelon) is rich in citrulline, which can enhance nitric oxide production, thereby improving testicular blood flow and function [20]. The antioxidant effects of garlic and onion further support cellular integrity and tissue regeneration [14,15]. The findings suggest that the combined juice extracts possess androgenic and spermatogenic enhancing effects in diabetic rats, possibly via antioxidant mechanisms and improved testicular microenvironment. This indicates their potential as natural therapeutic agents for managing diabetic reproductive dysfunction. However, limitations include the short duration of the study and lack of hormonal profiling (e.g., testosterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH)) to elucidate mechanisms fully. Future studies should include hormonal assays, longer treatment periods, and exploration of molecular pathways involved.

CONCLUSION

The combined juice extracted from *Allium cepa, Allium sativum, and Citrullus lanatus* significantly ameliorated diabetes-induced impairments in semen parameters and testicular histology in male Wistar rats. These findings support their potential as natural, safe, and effective interventions for improving reproductive health in diabetic males. Further research is warranted to confirm these effects and elucidate the precise mechanisms involved.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Michael Nsikak Umoh conceived and designed the study. Effiong M. Oku, Eru E. Mba, Kelechi C. Uruakpa, Cyril A. Agbor, Victor K. Uno, Eric A. Agim. Emmanuel I. Odom and Godwin A. Bekwapu collected and analyzed the data. Emmanuel I. Odom wrote the manuscript. All authors read and approved the final manuscript for publication.

Ethical Approval

Ethical approval was obtained for all studies.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Use of Artificial Intelligence/Large Language Models

No artificial intelligence (AI)-based systems were used for any portion of this work.

Use of Research Reporting Tool

No research reporting tool was used during the study or drafting of the manuscript.

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