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Research Article

Protective effects of *Cassia fistula* on epididymal histopathology, oxidative stress and reproductive performance in streptozotocin-induced Diabetic male Wistar rats

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Abstract

Background: Diabetes mellitus (DM) negatively affects normal sperm function and male fertility. Medicinal plants, known for their rich antioxidant properties, can help mitigate male infertility induced by diabetes. Objective: This research is designed to explore the ameliorative effects of ethanolic (70%) extract of *Cassia fistula* Linn. pods on epididymal histopathology, oxidative stress, sperm quality, and fertility in diabetic rats. Materials and Methods: Type-I diabetes was induced in male Wistar rats by administering a single injection (i.p.) of streptozotocin (60 mg/kg body mass). The diabetic rats were then administered oral doses of *Cassia fistula* pod extract at different amounts (100, 250, and 500 mg/kg body weight per day) for a duration of 60 days. The outcomes were compared to those of diabetic rats administered the antidiabetic drug glibenclamide (5 mg/kg body weight per day). Sperm quality (count, motility, and viability), lipid peroxidation, and markers of antioxidant defense (catalase, glutathione, superoxide dismutase and ascorbic acid) in the epididymis were analyzed. Additionally, epididymal histopathology, fertility index, and litter size were assessed. Results: The oral administration of *Cassia fistula* pod extract or glibenclamide in diabetic rats notably improved sperm vitality, fertility rate, and progeny number. The treatment also enhanced epididymal antioxidant levels and reversed histopathological abnormalities in comparison to the untreated diabetic group. These outcomes were similar to those seen with the standard glibenclamide treatment. Conclusions: The findings of this study demonstrate that *Cassia fistula* pod extract exhibit potent antioxidant properties and provides significant benefits for alleviating epididymal dysfunction in diabetic male rats.

Keywords: Antioxidants, Epididymis, Fertility index, *Cassia fistula,* Sperm, Streptozotocin

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Introduction

Diabetes mellitus has emerged as one of the most significant societal health issues in today's society, with its prevalence rising rapidly worldwide. According to the International Diabetes Federation (IDF), approximately 536.6 million adults were living with diabetes globally in 2021, a figure projected to increase to around 783.2 million by 2045 (1). This state is marked by high blood glucose levels due to inadequate insulin secretion, dysfunctional insulin activity, or a blend of both. If left untreated, chronic hyperglycemia can cause damage, dysfunction, and failure of various organs. Among the secondary complications of diabetes, reproductive dysfunction is a significant concern, affecting males and females (2,3).

Evidence from both animal studies and clinical investigations strongly suggests that diabetes mellitus negatively impacts male and female reproductive function and fertility (4-7). Many studies have also shown a decline in both sperm count and quality (8-11) as well as reduction in fertility (10-12). Insulin therapy in diabetic rats has been shown to bring sperm count and motility back to baseline levels (13). Research on STZ-induced diabetic rats has shown that diabetes causes degeneration and shrinkage in the epididymis. Nevertheless, insulin treatment in these rats has been shown to mitigate these adverse effects (14-16). Insulin dependent diabetes mellitus women had fewer pregnancies and fewer births per pregnancy than non diabetic subjects (17).

Although the impact of diabetes on male reproductive health has been extensively researched, the precise mechanisms behind reproductive dysfunction in males remain unclear. Numerous investigations have demonstrated that high blood glucose levels leads to the overproduction of reactive oxygen species (ROS) and free radical, surpassing the organism's cellular antioxidant defense capacity and disrupting the cellular reduction-oxidation balance (18-19). Oxidative stress may be lessened by taking antioxidant supplements. Natural antioxidants found in medicinal plants and herbs are abundant and can be used to treat diabetes and its consequences (20-22).

In India, a large number of indigenous plants have been utilized for treating diabetes and its complications since the time of Charaka and Sushruth (23). Review of literature reveals that various plant extracts and their bioactive components such as

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alkaloids, terpenoids, tennins, polysaccharides, polyphenolic compounds, flavonoids exhibit ameliorative impacts on oxidative stress and diabetic complications (20-21). Furthermore, many medicinal plants or bioactive phytochemicals has been documented in restoring male reproductive health in diabetic animal models by reducing oxidative stress and/or enhancing blood glucose levels. This emphasizes the significance of phytotherapy as an effective approach to managing diabetes and addressing related male reproductive dysfunctions (22-24).

Cassia fistula Linn (Family–Caesalpiniaceae) is a native therapeutic plant and grown across India as a ornamental tree. In traditional medicine, virtually every part of *Cassia fistula* is utilized for the treatment of diverse ailments. The pods of plant are used as abortifacient, anodyne, anti-bilious, antidiabetic, anti-inflammatory, antipyretic, astringent, depurative, diuretic, emollient, purgative and tonic (25-29). Reports indicate that the pod extract of *Cassia fistula* exhibits minimal toxicity in mice, with an LD50 of 6600 mg/kg body weight (30).

Phytochemical research has revealed that the pods of *Cassia fistula* are abundant in vital minerals like potassium (K), calcium (Ca), iron (Fe), and manganese (Mn), alongside amino acids such as aspartic acid, glutamic acid, and lysine. Additionally, the pods are known to have various phytochemicals, including rhein, quercetin dehydrate, kaempferol, dihydrokaempferol, (+) catechin, (-) epiafzelechin, 3B-hydroxy-17-norpimar-8(9)-en-15-one, 1,8-dihydroxy-3-anthraquinone carboxylic acid, 3-formyl-1-hydroxy-8-methoxy anthraquinone, dimeric proanthocyanidin CFI, and 1,8-dihydroxy-3-methylanthraquinone (31-34).

The hypoglycemic effect of *Cassia fistula* pod extract has been demonstrated in hyperglycemic rats induced with streptozotocin (STZ) (35-38). Furthermore, in-vitro and/or animal model studies have also reported the antioxidant activity of *Cassia fistula* pods/ fruit extract (35,39-40).

To the best of our knowledge, the impact of *Cassia fistula* pods extract on diabetes-induced reproductive dysfunctions has been documented as minimal. Therefore, this current study aims to assess the ameliorative effects of the *Cassia fistula* pods extract on diabetes induced male infertility, particularly focusing on epididymal dysfunctions and fertility.

Materials and Methods

Collection of plant and preparation of extract

The pods of *Cassia fistula* were gathered from the University of Rajasthan (UOR) campus between the months of April-June. The plant specimen was verified by botanist of herbarium, UOR, Jaipur (Voucher No. RUBL21057). The fully developed pods of the plant were air-dried in a shelter, and then the dried pods were ground using an electric grinder. The obtained powder was blended with ethanol (70%) and left to stand for twenty-four hours at ambient temperature. The mixture underwent extraction using a Soxhlet apparatus at a temperature of 60-70°C. High-temperature and prolonged heating are avoided to prevent the decomposition of phytochemicals. Following extraction, the mixture was strained through filter paper, and the resulting filtrate was dried in a drying oven at 40°C. The resulting residue was then stored in an airtight container in a refrigerator until needed.

Animals

In this study, male rats of the Wistar strain (*Rattus norvegicus*), weighing between 170 and 210 grams, were selected. They were housed in polypropylene cages in the department's animal facility (1678/Go/ReBi/S/2012/CCSEA) under standard laboratory conditions. The rats were given access to water and a nutritious pellet-based diet. Animal handling adhered to the protocols established by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Approval for the research was obtained from the Institutional Animal Welfare Committee.

Inducing diabetes

Diabetes mellitus was induced in rats that had fasted overnight by administering a single injection (i.p.) of STZ at a dose of 60 mg/ kg, dissolved in a freshly prepared citrate buffer (pH 4.5). Seven days post-STZ injection, fasting blood glucose levels were assessed using blood samples collected from the tail vein of the rats to confirm the presence of diabetes. Rats exhibiting fasting blood glucose levels above 250 mg/dl were categorized as diabetic and included in the experiment.

Experimental protocol

The test rats were separated into six categories, with each group consisting of six animals, and all were administered oral treatment for 60 continuous days. The treatment period for the current experiment was determined based on the duration of the spermatogenic cycle in rats, which spans approximately 50–52 days, along with an additional week to account for the time sperm remain in the epididymis (41-42). Based on the LD_{50} of *Cassia fistula* pods extract and findings from previous antidiabetic studies of various parts of plant, different doses were selected for this study (30, 43-44). The groups were organized as follows:

Group I: Normal control rats received 0.5 ml of distilled water, daily.

Group II: Untreated diabetic rats received 0.5 ml of distilled water, daily.

Group III: Diabetic rats received a low dose of extract (100 mg/ kg, BW) in 0.5 ml.

Group IV: Diabetic rats received a medium dose of extract (250 mg/kg, BW) in 0.5 ml.

Group V: Diabetic rats received a high dose of extract (500 mg/kg, BW) in 0.5 ml.

Group VI: Diabetic rats received 0.5 ml of glibenclamide at a dose of 5 mg/kg, BW.

Sperm parameters

Sperm parameters were carried out following the completion of the 60-day treatment. Sperm motility as well as concentration was assessed by carefully dissecting the cauda epididymis (100 mg) and placing it in 2.0 ml of sterile saline solution (0.9% NaCl, 37°C). The resulting mixture was filtered through a nylon mesh to separate the sperm from the surrounding tissue. Sperm concentration was quantified using a Neubauer chamber of a hemocytometer under a light microscope (X100) and reported as millions per milliliter of suspension. Motility was determined by observing both active and immobile sperm under the same light microscope (X100) and calculated as a percentage. To assess sperm viability, one drop of 1% eosin-y and 10% nigrosin was added to a microcentrifuge tube, followed by a sperm suspension drop and thoroughly mixed. A drop of this blend was placed on a glass slide and examined under a light microscope (X400). Dead sperm were identified by red staining, while live sperm remained unstained, and at least 200 cells were analyzed (45).

Fertility and litters size

To evaluate the reproductive outcome of the experimental rats, a mating trial was performed during the final 5 days of the treatment phase. Male rats were kept overnight with untreated, naturally cycling females (in proestrus or estrus) at a 1:2 ratio to facilitate copulation. The following morning, mating was confirmed by the presence of sperm or a copulatory plug in vaginal smears, which was regarded as the initial day of pregnancy. The females that showed sperm presence were subsequently separated and carefully observed until they gave birth. The count of live pups born on the delivery day was documented. Fertility was calculated by dividing the number of sperm-positive females by the total number of females, then multiplying the result by 100.

Epididymal lipid peroxidation and antioxidant markers

Epididymal tissue samples from rats in different groups were analyzed to estimate (TBARS), Superoxide dismutase (SOD), Reduced glutathione (GSH), Ascorbic acid (Vitamin C) and Catalase (CAT) (46-50). A minimum of six samples from each group were collected and averaged.

Histopathological study

Epididymal tissue specimens were initially preserved in Bouin's solution. After fixation, the tissues were washed with DW to eliminate any excess fixative. The samples were then dehydrated by passing through a series of graded alcohol solutions and cleared with xylene to remove any residual alcohol. The tissues were subsequently embedded in paraffin wax to provide structural integrity. Once embedded, the specimens were cut into thin sections, each 5 µm in thickness. These tissue sections were stained with hematoxylin and eosin for enhanced visualization. The prepared slides were examined using a light microscope, and images were taken with a Nikon digital camera connected to the microscope.

Statistical analysis

All data were evaluated and statistically analyzed using SPSS version 20.0 for Windows (SPSS Inc., Chicago, IL, USA). The results are presented as mean ± standard error of the mean (SEM) and were analyzed for variance. Statistical differences were determined using one-way analysis of variance (ANOVA) with Tukey's post hoc test. A p-value of less than 0.05 was deemed statistically significant.

Results

Sperm Parameters and Reproductive Performance

Alterations in fertility rate, sperm characteristics and litter size examined in the normal control and various experimental groups are presented in Table 1.

Streptozotocin-induced diabetic rats shows a marked (P≤0.001) decrease in sperm count, viability and motility in sperm collected from the cauda epididymis compared to the normal control group. Administering *Cassia fistula* extract at three different doses (100, 250, and 500 mg/kg body weight/day) led to a significant and dose-dependent rise in sperm count in diabetic rats (P≤0.05, P≤0.01, and P≤0.001, respectively) compared to the diabetic control group. Sperm motility showed considerable improvement (P≤0.01 and P≤0.001) in rats treated with the medium (250 mg/kg) and high (500 mg/kg) doses of *Cassia fistula* extract, while no significant improvement was seen in the group receiving the lowest dose. Likewise, the number of viable sperm significantly increased (P≤0.05 and P≤0.01) in the medium and high dose groups, but no significant change was noted in the lowest dose group. Diabetic rats administered glibenclamide also showed a marked (P≤0.001) enhancement in sperm count, viability and motility when compared to the diabetic control group.

STZ-induced diabetic control rats demonstrated a significant decrease in the fertility index (41.66%) relative to normal control rats (91.66%). Administration of *Cassia fistula* extract to diabetic rats at doses of (100, 250, and 500 mg/kg body weight/day) led to a dose-dependent enhancement in the fertility index, with values of 50%, 66.67%, and 75%, respectively. Likewise, diabetic rats administered with glibenclamide exhibited a considerable enhancement in the fertility index, reaching 83.33%.

The average litter size in diabetic control rats was significantly lower (P≤0.001) compared to normal control rats. Treatment with 250 and 500 mg/kg body weight/day of *Cassia fistula* extract led to a significant rise in mean litter size (P≤0.01 and P≤0.001, respectively) compared to the diabetic control group. However, no significant improvement was observed in diabetic rats treated with 100 mg/kg body weight of *Cassia fistula* extract. Diabetic rats receiving glibenclamide exhibited a marked (P≤0.001) rise in average litter size relative to the diabetic control group.

Table 1: Impact of *Cassia fistula* Extract on Cauda Epididymal Sperm Quality and Reproductive Outcomes in Streptozotocin-Induced Diabetic Rats

Treatment	Sperm count (Million/mL)	Sperm Motility (%)	Sperm Viability (%)	Fertility (%)	Litter size
Group I Normal Control	47.59± 2.50	74.87± 3.52	81.13± 3.64	91.66 (11/12)	9.36± 0.33
Group II Diabetic Control	18.68± 1.57***	36.32± 2.74***	44.25± 3.45***	41.66 (5/12)	4.40± 0.40***
Group III Diabetic+ Extract (100mg/kg b.wt.)	28.90± 1.41 [a]	44.12± 4.02 [ns]	53.92± 3.23 [ns]	50.00 (6/12)	5.33± 0.42[ns]
Group IV Diabetic+ Extract (250mg/kg b.wt.)	33.08± 2.62 [b]	53.98± 2.08 [b]	61.62± 2.79 [a]	66.67 (8/12)	6.38± 0.32[b]
Group V Diabetic+ Extract (500mg/kg b.wt.)	38.58± 2.95 [c]	58.92± 3.85 [c]	64.87± 4.69 [b]	75.00 (9/12)	7.44± 0.29[c]
Group VI Diabetic+ Glibenclamide (5mg/kg b.wt.)	40.72± 2.64 [c]	60.63± 3.50 [c]	69.02± 3.67 [c]	83.33 (10/12)	7.90± 0.38[c]

"Values represent mean ± SEM (n=6), Level of significance: *** = P ≤ 0.001, diabetic control rats compared with normal control rats. ns = non significant; a = P ≤ 0.05 ; b = P ≤ 0.01; c = P ≤ 0.001, *Cassia fistula* extract or glibenclamide treated rats compared with diabetic control rats"

Lipid Peroxidation and Antioxidant Markers

The alterations in TBARS, CAT, SOD GSH, and ascorbic acid levels in various tissue samples from the various experimental groups are presented in Figures 1 to 5.

Lipid peroxidation was evaluated by quantifying the levels of thiobarbituric acid reactive substances (TBARS). Diabetic control rats exhibited a significant (P≤0.001) rise in TBARS levels in the epididymal tissue in comparison to the normal control group. Conversely, a decrease in TBARS levels was observed in diabetic rats treated with *Cassia fistula* extract at all doses. The reduction was highly significant (P≤0.001) for the medium (250 mg/kg body weight) and high (500 mg/kg body weight) doses, while it showed a modest significant decrease (P≤0.05) for the low dose in comparison to the diabetic control group. Diabetic rats treated with glibenclamide also exhibited a marked reduction in TBARS levels in the epididymis relative to the diabetic control rats.

STZ-induced diabetic rats showed a significant reduction (P≤0.001) in the activities of SOD and catalase, along with decreased amount of ascorbic acid and GSH in the epididymal tissues when compared to the normal control group. Diabetic rats administered with *Cassia fistula* extract at doses of 250 and 500 mg/kg body weight/day for 60 days exhibited a marked increase (P≤0.01 and P≤0.001, respectively) in SOD and catalase activities, as well as elevated GSH and ascorbic acid levels in the epididymis relative to the diabetic control rats. However, diabetic rats given the low dose (100 mg/kg body weight) of *Cassia fistula* extract showed a modest but significant rise (P≤0.05) in GSH levels, with no significant changes observed in the activities of SOD and catalase or in ascorbic acid levels. Additionally, diabetic rats treated with glibenclamide demonstrated a significant (P≤0.001) improvement in the activities of both SOD and catalase, along with increased levels of GSH and ascorbic acid in the epididymal tissue.

Figure 1: Effect of *Cassia fistula* on lipid peroxidation. "Level of significance: *** = P ≤ 0.001, diabetic control rats compared with normal control rats. ns = non significant; a = P ≤ 0.05 ; b= P ≤ 0.01; c = P ≤ 0.001, *Cassia fistula* extract or glibenclamide treated rats compared with diabetic control rats"

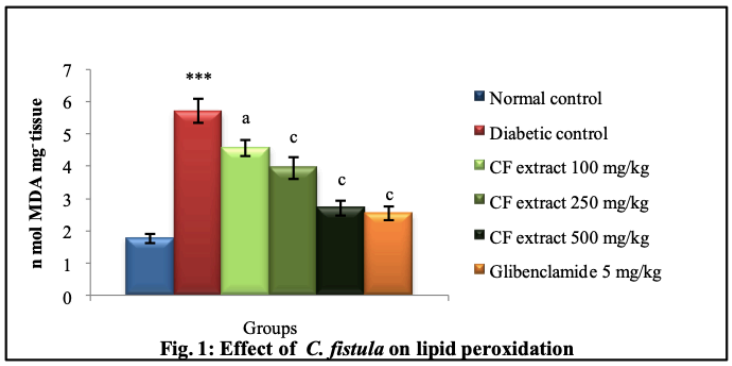


Fig. 1: Effect of *C. fistula* on lipid peroxidation

Figure 2: Effect of *Cassia fistula* on activity of SOD. "Level of significance: *** = P ≤ 0.001, diabetic control rats compared with normal control rats. ns = non significant; b = P ≤ 0.01; c = P ≤ 0.001, *Cassia fistula* extract or glibenclamide treated rats compared with diabetic control rats"

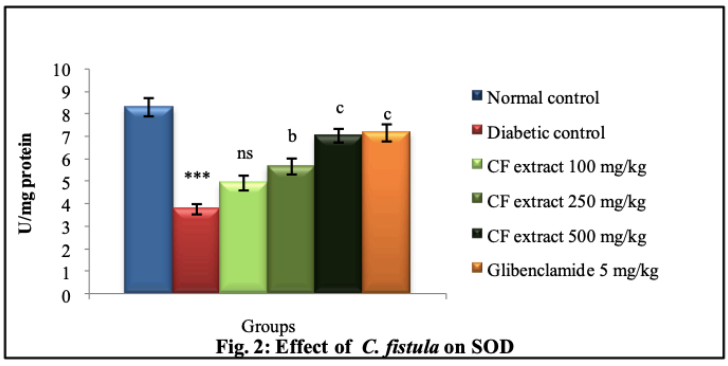


Fig. 2: Effect of *C. fistula* on SOD

Figure 3: Effect of *Cassia fistula* on activity of Catalase. "Level of significance: *** = P ≤ 0.001, diabetic control rats compared with normal control rats. ns = non significant; b = P ≤ 0.01; c = P ≤ 0.001, *Cassia fistula* extract or glibenclamide treated rats compared with diabetic control rats"

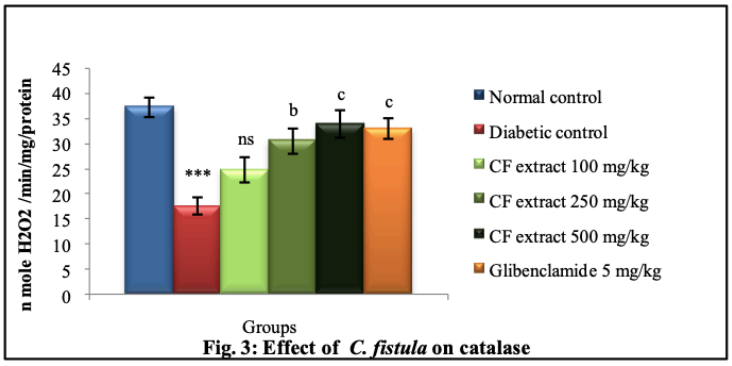


Fig. 3: Effect of *C. fistula* on catalase

Figure 4: Effect of *Cassia fistula* on level of GSH. "Level of significance: *** = P ≤ 0.001, diabetic control rats compared with normal control rats. a = P ≤ 0.05 ; b = P ≤ 0.01; c = P ≤ 0.001, *Cassia fistula* extract or glibenclamide treated rats compared with diabetic control rats"

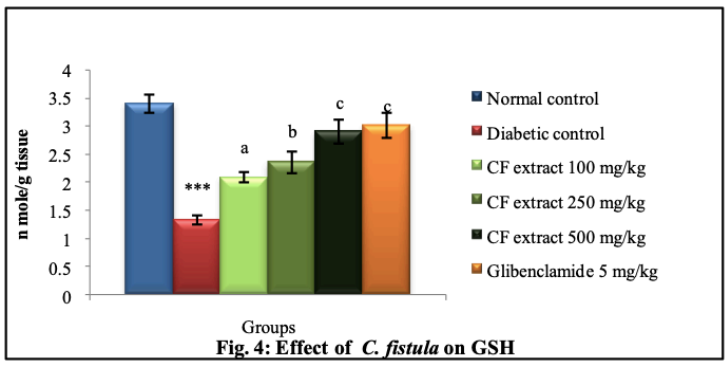
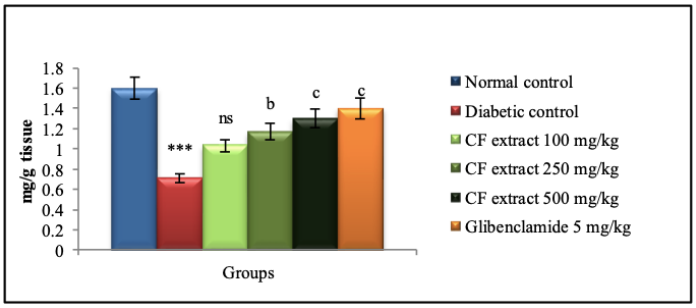


Fig. 4: Effect of *C. fistula* on GSH

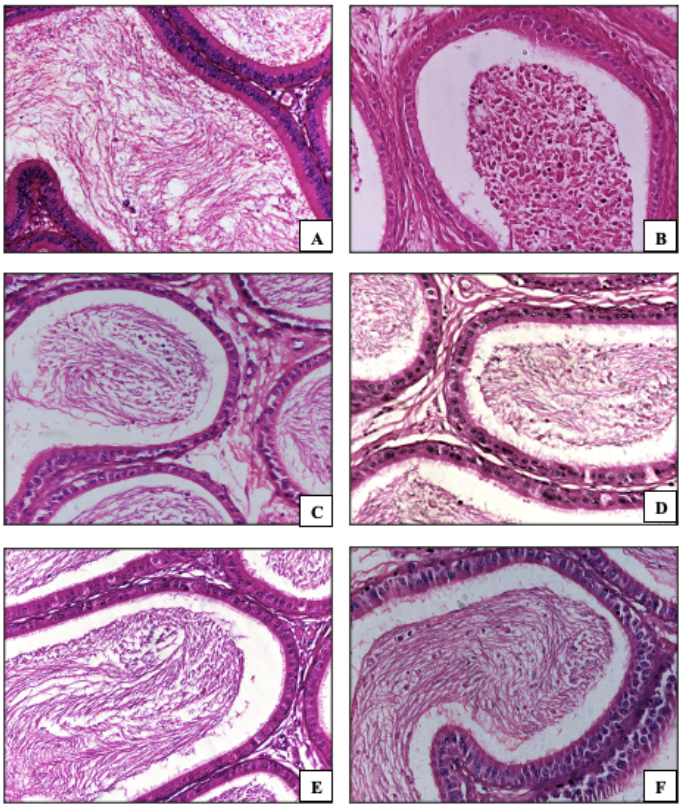
Figure 5: Effect of *Cassia fistula* on level of Ascorbic acid. "Level of significance: *** = P ≤ 0.001, diabetic control rats compared with normal control rats. ns = non significant; b = P ≤ 0.01; c = P ≤ 0.001, , *Cassia fistula* extract or glibenclamide treated rats compared with diabetic control rats"



Histopathology

Histological examination of the epididymis in normal control rats revealed large tubules with spacious lumens, lined by ciliated pseudostratified columnar epithelium. The lumens were densely populated with sperm cells (Figure 6A). In contrast, the epididymis of diabetic control rats showed significant shrinkage in tubule size, reduced epithelial cell height, and an increase in intertubular tissue. Most tubules lacked sperm cells (Figure 6B). Diabetic rats treated with a low dose of *Cassia fistula* extract (100 mg/kg body weight/day) exhibited minor mitigation of degenerative changes in the cauda epididymis, with a few sperm cells and debris present within the lumens (Figure 6C). Rats given a moderate dose of *Cassia fistula* extract (250 mg/kg body weight/ day) showed an improvement in epithelial cell height, a higher count of spermatozoa in the lumens, and a reduction in the intertubular tissue (Figure 6D). Diabetic rats receiving the high dose of *Cassia fistula* extract (500 mg/kg body weight/day) displayed significant restoration of epididymal structure, including larger tubules, increased epithelial cell height, and a substantial presence of sperm cells within the lumens (Figure 6E). Treatment with the standard drug, glibenclamide, led to nearly normal epididymal architecture. The tubules were lined with tall epithelial cells and long stereocilia, the lumens were filled with abundant sperm, and the intertubular tissue was markedly reduced compared to diabetic control rats (Figure 6F).

Figure 6: Photomicrographs of the cauda epididymis:



(A) Normal control rats exhibiting intact histoarchitecture. (B) Diabetic control rats demonstrating tubule shrinkage, thickened fibromuscular layer, and sperm debris in the tubular lumen. (C) Diabetic rats treated with *Cassia fistula* extract (100mg/kg b.wt.) showing mild preservation of degenerative and atrophic changes. (D) Diabetic rats treated with *Cassia fistula* extract (250mg/kg b.wt.) presenting moderate improvement in histoarchitecture. (E) Diabetic rats treated with *Cassia fistula* extract (500mg/kg b.wt.) displaying significant restoration of histomorphology. (F) Diabetic rats treated with glibenclamide showing nearly normal histoarchitectural appearance (H & E x200).

Discussion

The epididymis plays a crucial role in male fertility, functioning not only as a passageway but also as an active participant in producing a fertile ejaculate. It is responsible for the transport, concentration, maturation, and storage of spermatozoa (51). Key sperm parameters, including sperm count, motility, morphology, and viability, are essential indicators of male fertility. Evaluating these parameters offers valuable insights into the impact on male reproductive health and fecundity (52).

In the present investigation, a notable reduction in sperm count, viability and motility in the cauda epididymis was observed when compared to the normal control group. These results are consistent with earlier studies that have reported comparable reductions in sperm count, viability and motility in STZ-induced diabetic rats or mice, (12,53-54) and also in diabetic subject (55-57). The ideal concentration of testosterone is crucial for the proper progression of spermatogenesis and the preservation of sperm structure and function (58). Insulin deficiency in diabetic rats has shown low levels of testosterone due to disturbance of leydig cell function via impairment of hypothalamus- pituitary ganadal axis. Studies have shown a positive relationship between testosterone levels and sperm motility (59). Another contributing factor to the reduced sperm motility observed in diabetic rats could be the heightened production of free radicals and oxidative stress, which leads to increased lipid peroxidation. Increased lipid peroxidation inside the male reproductive system is linked to detrimental effects on sperm count, motility, morphology, and membrane integrity, ultimately resulting in infertility (60-61).

The results of the current study demonstrated that administration of *Cassia fistula* extract significantly enhanced sperm count, viability and motility in diabetic rats in a dose-dependent fashion. The beneficial effects of the extract on sperm characteristics may be attributed to its potent bioactive compounds, which helped regulate blood glucose levels, boost antioxidant activity, and restore hormonal balance, thus supporting spermatogenesis in the testes. These findings align with prior research, which also reported improvements in blood sugar levels and male reproductive health in diabetic rats treated with antidiabetic plant extracts (11,22,23).

In the present study, diabetic control rats showed a significant decrease in fertility percentage and average litter size. These findings align with previous research that indicated a significant decline in fertility among diabetic animal (11,62-64). The reduction in fertility observed in diabetic rats may be due to disrupted sperm development, lower sperm count and motility, along with a rise in sperm defects (65). Diabetes also exacerbates oxidative stress and lipid peroxidation, leading to damage in sperm nuclear and mitochondrial DNA (66-68). Such DNA damage in male germ cells is linked to a decrease in fertilization success, abnormal pre-implantation embryonic development, and an elevated risk of miscarriage (69).

Treatment with *Cassia fistula* extract or glibenclamide led to a marked enhancement in fertility rates and average litter size in diabetic rats. These improvements may be attributed to the positive effects on sperm parameters, elevated testosterone levels, restoration of antioxidant balance, and better regulation of blood glucose levels. These findings are in line with earlier research that reported similar enhancements in fertility and litter size in diabetic rats treated with antidiabetic plant extracts (11,63,70). The results of the study demonstrated a significant rise in lipid peroxidation, as evidenced by increased TBARS levels, alongside a substantial decrease in antioxidant markers such as SOD, GSH, CAT and ascorbic acid in the epididymal tissues. These results align with earlier research that documented comparable reductions in antioxidant defense and higher TBARS levels in the epididymis of diabetic animals (64,71). The elevated TBARS levels in diabetic control rats suggest an inadequate antioxidant defense system to counteract the oxidative stress induced by ROS. This situation is further worsened by the naturally low antioxidant capacity of the epididymis (72).

Oxidative stress is a key factor in the development of diabetes and its related reproductive issues, ultimately leading to infertility (73,74). The observed decrease in antioxidant molecules may be due to their deactivation by reactive oxygen species (ROS) or the glycation of antioxidant enzymes. Moreover, the heightened oxidative stress in the epididymis could be connected to lower testosterone levels, which are in turn linked to reduced serum insulin levels. Treatment with *Cassia fistula* extract in diabetic rats remarkably boosted the activity of antioxidant enzymes (SOD and CAT) and elevated the levels of non-enzymatic antioxidants (GSH and ascorbic acid), while also reducing TBARS levels in the epididymis. This enhancement in antioxidant activity can be attributed to the potent antioxidant compounds present in the *Cassia fistula* extract.

Glibenclamide treatment also significantly reduced TBARS levels and enhanced the activities of key antioxidant enzymes such as SOD and CAT, along with an increase in the levels of GSH and ascorbic acid. The observed reduction in oxidative stress in glibenclamide-treated diabetic rats in the present study is consistent with the findings of Chatterjee et al., 2013 (75). Glibenclamide's antioxidant effect may be attributed to its ability to improve overall metabolic control in diabetes, particularly through the reduction of blood glucose levels by stimulating insulin secretion. This is achieved via inhibition of ATP-sensitive K^+ (KATP) channels in pancreatic β-cell membranes, leading to depolarization, activation of voltage-gated Ca^{2+} channels, and subsequent insulin release (76). Our previously published data from the same study demonstrated that glibenclamide more effectively regulated blood glucose levels compared to the highest dose of *Cassia fistula* pod extract in diabetic rats. However, both treatments exhibited comparable antioxidant activity, suggesting that the *Cassia fistula* extract mitigates oxidative stress not only by glycemic control but also through the intrinsic antioxidative properties of its phytoconstituents (35-36).

Administration of streptozotocin in rats led to significant degenerative and atrophic alterations in the epididymis, marked by a decrease in tubular diameter and epithelial cell height, an expansion of intertubular fibromuscular tissue, damaged or shortened stereocilia, and lumens filled with sperm debris, devoid of spermatozoa. These findings correspond with previous research that described comparable degenerative alterations in the epididymis of diabetic rats (53,77-79). The near-complete absence of spermatozoa in the lumens suggests a disruption in spermatogenesis within the testes, likely attributed to reduced testosterone levels. Furthermore, the depletion of antioxidant defense systems, resulting from hyperglycemia and lowered insulin levels, likely contributes to the structural and functional damage observed in the epididymis of diabetic rats.

Treatment with *Cassia fistula* extract or glibenclamide in diabetic rats markedly and dose-dependently restored the cauda epididymal histoarchitecture. This was evidenced by larger tubules lined with columnar epithelial cells bearing long stereocilia, lumens containing a substantial number of spermatozoa, and reduced intertubular stroma. These ameliorative effects may be attributed to increased serum insulin and testosterone levels, as well as the attenuation of oxidative stress caused by hyperglycemia. Consistent with the present study, previous research has also demonstrated the protective effects of medicinal plants with antidiabetic and antioxidant properties (78-80).

Conclusion

The finding from the present study demonstrated that ethanolic extract of *Cassia fistula* pods significantly ameliorate diabetes induced epididymal dysfunctions and infertility via multiple mechanism including increase insulin secretion, inhibition of lipid peroxidation, enhancement of the antioxidant's activity/ concentration and preservation of normal epididymal histology and sperm parameters. This research provides scientific validation for the traditional utilization of *Cassia fistula* pod in the treatment of diabetes mellitus. Additional investigation is required to isolate the specific bioactive phytoconstituent(s) responsible for this therapeutic activity.

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