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Ameliorative Effects of *Cassia fistula* Pod Extract on Testicular Histopathology, Oxidative Stress and Reproductive Hormone Levels in Streptozotocin Induced Diabetic Rats

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Diabetes mellitus (DM) has been linked to reproductive impairments. Medicinal plants have shown potential in alleviating diabetes-induced reproductive dysfunction in male. The Primary aim of the study was to assess the influence of an extract derived from *Cassia fistula* pod on reproductive hormone levels and testicular dysfunction in diabetic rats. A streptozotocin (STZ) dose (60 mg/kg b.wt.) was intraperitoneally (i.p.) injected to Wistar male rats to induce diabetes. 36 male rats were randomly assigned to six different groups: a healthy control group, a diabetic control group, three diabetic groups administered varying amount of *Cassia fistula* extract (100, 250, 500 mg/kg body weight per day), and a diabetic group receiving glibenclamide (5 mg/kg body weight per day). The treatment was given every day for 60 consecutive days. Levels of reproductive hormones including follicle-stimulating hormone (FSH), testosterone and luteinizing hormone (LH), along with oxidative stress in testicular tissue, were assessed. Histomorphometric and histopathological alterations in the testes were also examined. Diabetic control group exhibited significant decline in testicular weight, the testicular germ cells population, seminiferous tubular diameter and reproductive hormones like testosterone, FSH and LH as compared to control rats. Additionally, significant rise in lipid peroxidation (TBARS) alongside a simultaneous reduction in SOD and CAT activities as well as ascorbic acid and glutathione levels within the testicular tissues were observed compared to control rats. The administration of *Cassia fistula* extract or glibenclamide via oral route in diabetic rat led to improvements in serum insulin and reproductive hormone concentrations. Additionally, a reversal of histopathological and histomorphometric changes was noted relative to the diabetic reference group. Furthermore, the administration with the extract decreased lipid peroxidation and increased antioxidant levels in testicular tissue in comparison to the untreated diabetic rats. The outcomes of this research reveal that the hydroalcoholic extract from *Cassia fistula* pod exhibits significant antioxidant activities and can also modulate testicular dysfunction in diabetic male rats.

Keywords: Antioxidant activity; Cassia fistula; Histopathology; Oxidative stress; Streptozotocin; Testosterone; Testicular dysfunction.

Today diabetes mellitus is amongst the most significant public health concerns, and its prevalence is rising rapidly worldwide. As estimated by the IDF, approximately 536.6 million adults globally were living with diabetes in 2021, with the figure expected to rise to nearly 783.2 million by 2045.[1] Diabetes mellitus is marked by raised blood sugar level due to inadequate insulin production, ineffective insulin action, or both. Without treatment, persistently elevated

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blood sugar can lead to damage, dysfunction, and eventual failure of multiple organs. Reproductive dysfunction emerges as a significant secondary complication of diabetes mellitus in both males and females.[2,3]

Evidence from both animal studies and clinical investigations strongly suggests that diabetes mellitus negatively impacts male reproductive function and fertility.[4] Research conducted on experimental diabetic rats has revealed a decrease in testosterone and gonadotropin levels.[5,6] Numerous studies also reported that diabetes mellitus is associated with testicular dysfunctions viz. A decline in testicular weight, degradation of germ cells and seminiferous epithelium height, along with shrinkage and degenerative alterations in the testis has been observed.[7,8]

Research indicates oxidative stress as a crucial factor and as the primary cause of testicular impairment, ultimately leads to male hypogonadism, bad sperm quality, and infertility.[9,10] Both the sperm and testes generate insulin, and insulin production within the testes seem to be affected by diabetes. However, the negative impact of diabetes could also arise from disrupted signaling of insulin in the testes and brain, systemic consequences of deficit in insulin, or a combination of both factors.[11,12] Numerous investigations have demonstrated that high blood glucose levels led to an excess of reactive ROS and free radicals to be produced, surpassing the organism's cellular antioxidant defense capacity and disrupting the cellular reduction-oxidation balance.[13,14] 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.[14-16]

In India, a significant variety of plants have been utilized for treating diabetes and its complications since the time of Charaka and Sushruth.[15] Review of literature reveals that various plant extracts and their bioactive components exhibit ameliorative impacts on oxidative stress and diabetic complications. These natural products-based phytochemicals target increased insulin secretion, regeneration/protection of insulin secreting β-cells, some have extra pancreatic effect by acting directly on tissue like liver, muscles etc. and regulation of glycolysis and gluconeogenesis, inhibit carbohydrate digestion and absorption by blocking α-glucosidase activity in the intestine.[17,18]

Review of literature reveals that various plant extracts and bioactive compounds have shown beneficial effects in maintaining blood glucose levels, strengthening antioxidant defense mechanisms, and improving reproductive health in male diabetic rats. This emphasizes the significance of phytotherapy as a possible approach for managing diabetes and related male reproductive disorders.[19,20]

Cassia fistula Linn (Family–Caesalpiniaceae) is an indigenous medicinal plant and cultivated throughout India as an ornamental tree. This species is native to the Indian Subcontinent and Myanmar. It is a tree that predominantly thrives in seasonally dry tropical regions. It serves multiple purposes, including use as animal feed, traditional medicine, and poison. Additionally, it has environmental and social applications, and is utilized for food and fuel. 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, antibilious, antidiabetic, anti-inflammatory, antipyretic, astringent, depurative, diuretic, emollient, purgative and tonic. Phytochemical research has revealed that *Cassia fistula* pods are an abundant source of vital nutrients such as calcium (Ca), potassium (K), iron (Fe), and manganese (Mn). The pod of *Cassia fistula* are known to contain various phytochemicals, such as 1,8-dihydroxy-3-anthraquinone carboxylic acid, rhein, 3-formyl-1-hydroxy-8-methoxy anthraquinone, 3B-hydroxy-17-norpimar-8(9)-en-15-one, quercetin dehydrate, dimeric proanthocyanidin CFI, (+) catechin, (-) epiafzelechin, dihydrokaempferol, kaempferol and 1,8-dihydroxy-3-methylanthraquinone.[21-24]

The hypoglycemic impact of *Cassia fistula* extract has been shown in diabetic rats induced with STZ.[25-27] Furthermore, in-vitro studies and/or animal model studies have also reported the antioxidant activity of *C. fistula* pod/fruit pulp extract.[25,28,29]

As far as we are aware, research on the influence of *Cassia fistula* pod extract on diabetes-induced reproductive dysfunctions has been found to be very limited. Therefore, the objective of the current study is to assess the ameliorative effects of the *Cassia fistula* pod extract on male reproductive

dysfunctions, particularly focusing on testicular dysfunctions and serum levels of reproductive hormones.

MATERIALS AND METHODS

Plant collection and preparation of its extract

The pods of *Cassia fistula* were gathered from the University of Rajasthan campus between the months of April-June. The plant specimen was verified by botanist of herbarium, 'University of Rajasthan', Jaipur (Voucher No. RUBL21057). The mature pods of the plant were dried in shed then the dried pods were powder by using an electric grinder. The obtained powder was mixed with 70% ethanol and left undisturbed for 1 day at room temperature. The mixture underwent extraction using a Soxhlet apparatus at a temperature of 60°C. High-temperature and prolonged heating are avoided to prevent the decomposition of phytochemicals. After extraction, the solution underwent filtration using filter paper, and obtained filtrate was subsequently dried in the oven at a temperature of 40°C. The thick brown paste yield from *Cassia fistula* was approximately 33 g (13.2% of the dried powder). This residue was stored in a sealed airtight bottle within a refrigerator until needed.

Animals

In this study, Wistar strain male rats (*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 water and nutritious pellet diet. Animal handling was conducted in accordance with the standards established by the "Committee for the Purpose of Control and Supervision of Experiments on Animals" (CPCSEA). The research received approval from the Institutional Animal Ethics Committee.

Inducing diabetes

Rats that had fasted overnight were induced with diabetes mellitus by a single intraperitoneal injection of STZ at a dosage of 60 mg/kg, mixed with freshly prepared citrate buffer (pH 4.5). Seven days following the STZ administration, fasting blood glucose levels were assessed by collecting samples of blood from the tail vein of rats that had been fasted overnight. Rats with fasting blood glucose concentrations above 250 mg/dl were classified as diabetic and selected for inclusion in the study.

Experimental protocol

The animals were split into six groups, each group containing 6 rats, and all were treated orally for 60 consecutive days. The groups were organized as follows:

Group I: Normal control rats were given 0.5 ml of DW daily.

Group II: Untreated diabetic rats were given 0.5 ml of DW daily.

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

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

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

Group VI: Diabetic rats were given glibenclamide at a dose of 5 mg/kg in 0.5 ml.

Testicular weight and Serum hormone profiles

At the conclusion of the intervention period, all rats that had fasted overnight were weighed and euthanized under light ether anesthesia for autopsy. The testes were removed, rinsed with ice-cold saline, cleaned of any residual fat and blood clots, and then weighed using an electronic balance. Samples of blood were collected via direct puncture in heart, and serum was isolated by centrifuging the blood at 3000 rpm for ten minutes. The concentrations of serum testosterone, FSH, insulin, and LH were assessed using a chemiluminescent immunoassay.

Testicular lipid peroxidation and antioxidant markers

Testicular tissue samples from rats in various groups were utilized to measure Lipid peroxidation (TBARS),[30] Reduced glutathione (GSH),[31] Superoxide dismutase (SOD),[32] AA (Vitamin C),[33] and Catalase (CAT),[34]. A minimum of six samples from each group were collected and averaged.

Histopathological study

For histopathological examination, testis tissue samples were initially preserved in Bouin's solution. After fixation, the samples were rinsed with distilled water (DW) to remove any excess fixative. Next, the tissues underwent dehydration by immersing them in a series of concentrated alcohol

solutions, then cleared with xylene to eliminate any remaining alcohol. The tissues were subsequently embedded in paraffin wax for structural stability. After embedding, the tissues were sliced into thin sections, each 5 µm thick. These sections were stained using eosin and hematoxylin for enhanced visualization. The prepared slides were observed under a light microscope, and images were clicked with a Nikon digital camera connected to the microscope.

Histomorphometric analysis

Light microscope, fitted with an ocular micrometer and calibrated using a stage micrometer, was utilized to assess seminiferous tubules diameter (STs) and the nuclear size of Leydig cells. At a magnification of X100, two perpendicular diameters were recorded for each seminiferous tubule (40 tubules per group). These measurements were then averaged to determine the seminiferous tubule diameter. For the Leydig cells, nuclear diameters were measured at X1000 magnification (100 cells per group), and the results were averaged to calculate the mean Leydig cell nuclear diameter.

True Counts = Crude Counts × (Section Thikness (µm) / (Section Thikness (µm) + Nuclear diameter (µm))

Stage VII of the seminiferous tubule cycle was used for the quantitative evaluation of reproductive cells, such as spermatogonia and pachytene spermatocytes, preleptotene spermatocytes, and round spermatids, per cross-section (50 circular tubules per group) at X400 magnification.[35] The nuclear diameter of various types of germ cells was measured by using an ocular micrometer. A correction factor was applied to determine the accurate number density of germ cells.[36]

Statistical analysis

The data was examined and processed using SPSS 20.0 software for Windows (SPSS Inc., Chicago, IL, USA). Results are displayed as the average ± standard error of the mean (SEM), with variation assessed. One-way analysis of variance (ANOVA) was utilized to evaluate statistical differences, followed by Tukey's post hoc analysis. A p-value below 0.05 was regarded as statistically significant**.**

RESULTS

Testicular weight

Compared to group I, the testicular weight of diabetic rats (group II) decreased and were statistically significant (P≤0.001). When *Cassia fistula* extract was given to diabetic rats at low, medium, and high dosages, the absolute testicular weight increased in a dose related and significant manner (P≤0.05, P≤0.01, and P≤0.001, respectively). Glibenclamide administration also results in a significant (P≤0.001) rise in the absolute testicular weight compared to the diabetic control group (group II) as illustrated in Figure 1.

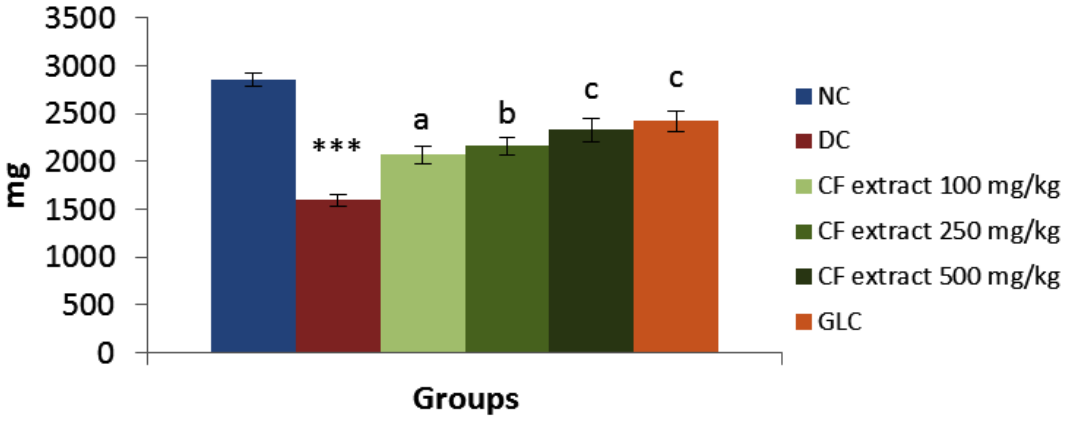

Fig. 1. Effect of extract of *'Cassia fistula'* (CF) on testis weight. "significance levels: ***=P≤0.001, comparing diabetic control rats to normal control rats; ns = not significant; a = P≤0.05; b = P≤0.01; c = P≤0.001, comparing *Cassia fistula* extract/glibenclamide-treated diabetic rats to the diabetic control group"

Serum hormones

Diabetic rats in group II showed a significant decrease (P≤0.001) in serum insulin, testosterone, FSH, and LH levels relative to the control rats in group I. Nevertheless, administering *Cassia fistula* extract at doses of 250 mg/kg as well as 500 mg/kg resulted in a significant increase in insulin levels (P≤0.05 and P≤0.001, respectively) in the diabetic rats. Likewise, there was a marked rise in testosterone, FSH, and LH levels (P≤0.01 and P≤0.001, respectively) relative to diabetic control group. Similar improvements were observed in group VI diabetic rats, with a significant rise (P≤0.001) in these serum hormones relative to group II diabetic rats (Table 1).

Lipid peroxidation

The testis tissue of group II diabetic rats revealed a noteworthy rise (P≤0.001) in levels of TBARS as compared to the group I normal rats. Diabetic rats which were given lower, medium, and higher doses of *Cassia fistula* extract exhibited a substantial dose responsive reduction (P≤0.05, P≤0.01, P≤0.001, respectively) in levels of TBARS in the testis in contrast to group II diabetic rats. Additionally, group VI rats displayed a remarkable (P≤0.001) fall in TBARS levels relative to group II diabetic rats (Figure 2).

Antioxidant Marker Parameters

Diabetic rats of group II exhibited a significant reduction (P≤0.001) in SOD, GSH,

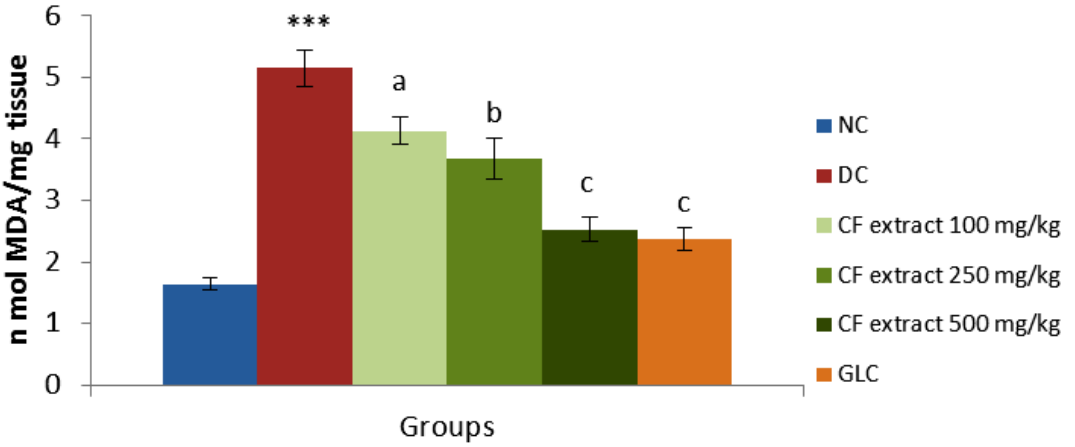

Fig. 2. Effect of extract of *'Cassia fistula'* (CF) on LPO level in testis. ''Significance levels: ***=P≤0.001, comparing diabetic control rats to normal control rats; ns = not significant; a = P≤0.05; b = P≤0.01; c = P≤0.001, comparing *Cassia fistula* extract/glibenclamide-treated diabetic rats to the diabetic control group''

Table 1. Effects of extract of '*Cassia fistula'* on levels of insulin and reproductive hormone

Groups	Insulin (µIU/mL)	Testosterone (ng/ml)	FSH (mIU/mL)	LH (mIU/mL)
Group I (Normal Control)	14.32±0.77	6.30±0.48	2.40±0.16	3.31±0.14
Group II (Diabetic Control)	4.18±0.29***	1.89±0.16***	0.87±0.07***	1.10±0.08***
Group III (Diabetic+100mg/kg of Extract)	5.91±0.36 ^{ns}	3.02±0.19 ^{ns}	1.42±0.11 ^{ns}	1.65±0.13 ^{ns}
Group IV (Diabetic+250mg/kg of Extract)	6.98±0.44 ^{a}	3.58±0.24 ^{b}	1.67±0.14^{b}	2.02±0.16^{b}
Group V (Diabetic+500mg/kg of Extract)	9.18±0.66 ^{c}	4.60±0.33 ^{c}	1.95±0.15^{c}	2.64±0.18 ^{c}
Group VI (Diabetic+5mg/kg of Glibenclamide)	10.32±0.85 ^{c}	4.70±0.38 ^{c}	2.01±0.16 ^{c}	2.72±0.24 ^{c}

''The values have been expressed as mean ± SEM (n=6), with the following levels of significance: *** = P≤0.001, comparing diabetic control rats to normal control rats; ns = not significant; a = P≤0.05; b = P≤0.01; c = P≤0.001, comparing *Cassia fistula* extract/glibenclamide-treated diabetic rats to diabetic control rats''.

CAT and ascorbic acid levels in testicular tissue relative to group I normal rats. However, diabetic rats treated with medium (group IV) and high (group V) doses of *Cassia fistula* extract displayed a marked increase in SOD and CAT activities, likewise GSH and ascorbic acid levels ($P \leq 0.01$ and $P \leq 0.001$, respectively) as compared to group II diabetic rats. In group III, treatment with a low dose of *Cassia fistula* extract to diabetic rats exhibited a slight but marked increase in ascorbic acid levels. Additionally, group VI exhibited a significant improvement ($P \leq 0.001$) in all antioxidant markers (Figure 3-6).

Histomorphometric analysis

Table 2 presents changes in the nuclear diameter of Leydig cells and diameter of STs along with the population of testicular germ cells. In diabetic group II, there was a noteworthy ($P \leq 0.001$) decline in the average diameter of STs and Leydig cell nuclei relative to control group I. Administration of *Cassia fistula* extract in the medium dose (group IV) and high dose (group

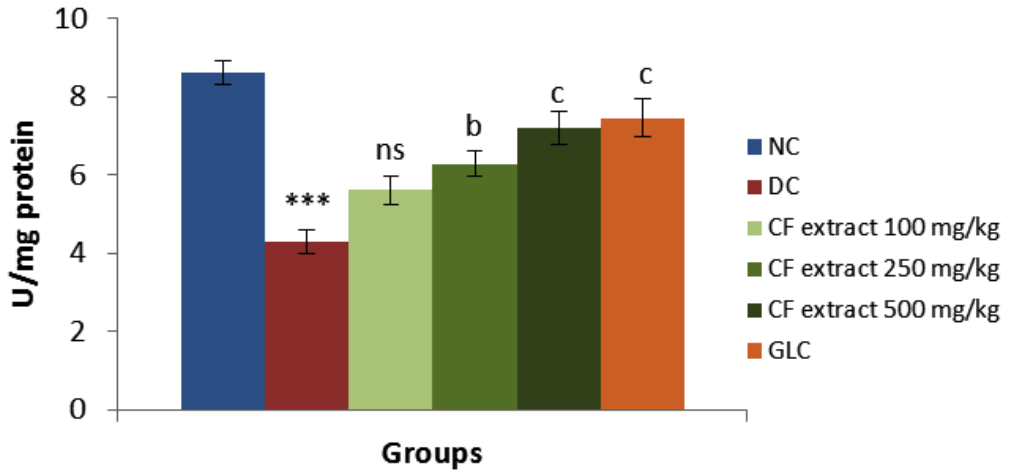

Fig. 3. Effect of extract of *'Cassia fistula'* (CF) on SOD activity in testis. "Significance levels: ***=$P \leq 0.001$, comparing diabetic control rats to normal control rats; ns = not significant; b = $P \leq 0.01$; c = $P \leq 0.001$, comparing *Cassia fistula* extract/glibenclamide-treated diabetic rats to the diabetic control group"

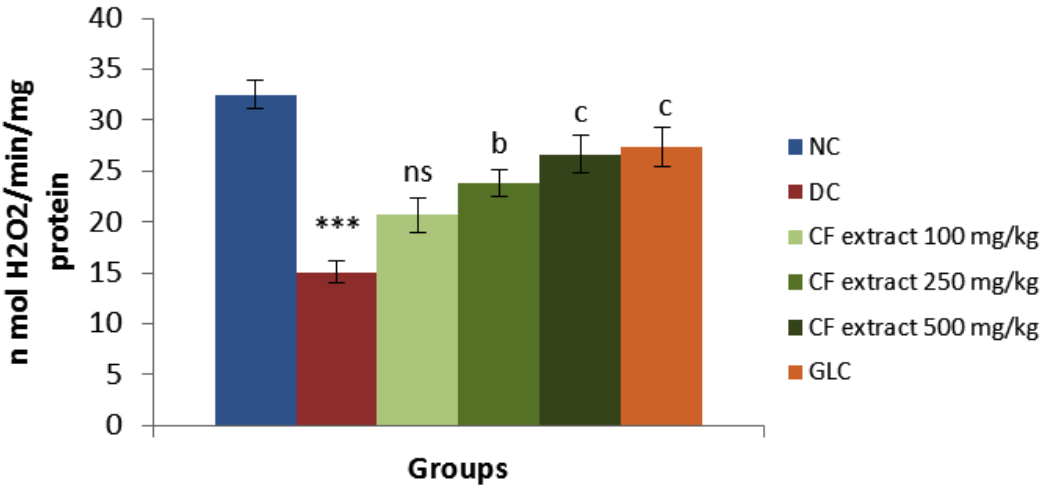

Fig. 4. Effect of extract of *'Cassia fistula'* (CF) on CAT activity in testis. "Significance levels: ***=$P \leq 0.001$, comparing diabetic control rats to normal control rats; ns = not significant; b = $P \leq 0.01$; c = $P \leq 0.001$, comparing *Cassia fistula* extract/glibenclamide-treated diabetic rats to the diabetic control group"

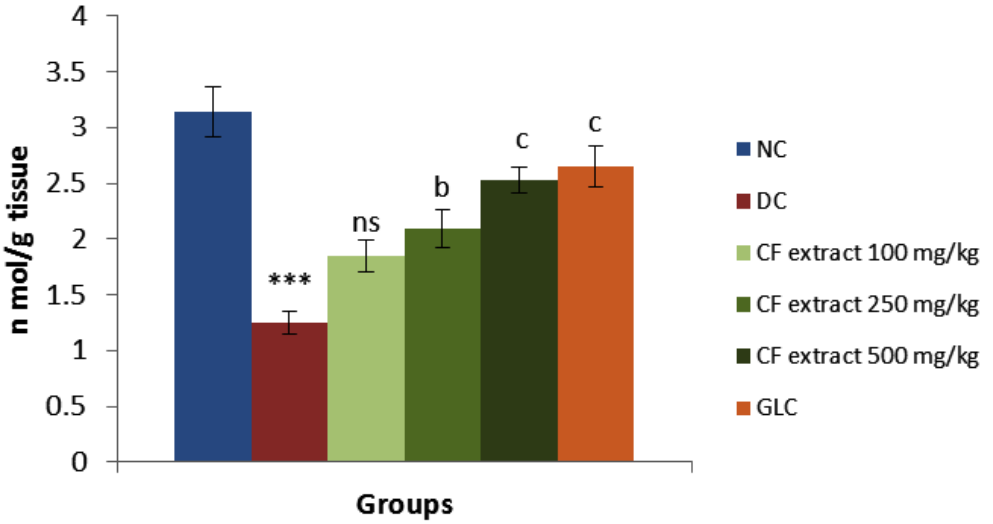

Fig. 5. Effect of extract of *'Cassia fistula'* (CF) on GSH level in testis. ''Significance levels: ***=P≤0.001, comparing diabetic control rats to normal control rats; ns = not significant; b = P≤0.01; c = P≤0.001, comparing *Cassia fistula* extract/glibenclamide-treated diabetic rats to the diabetic control group''

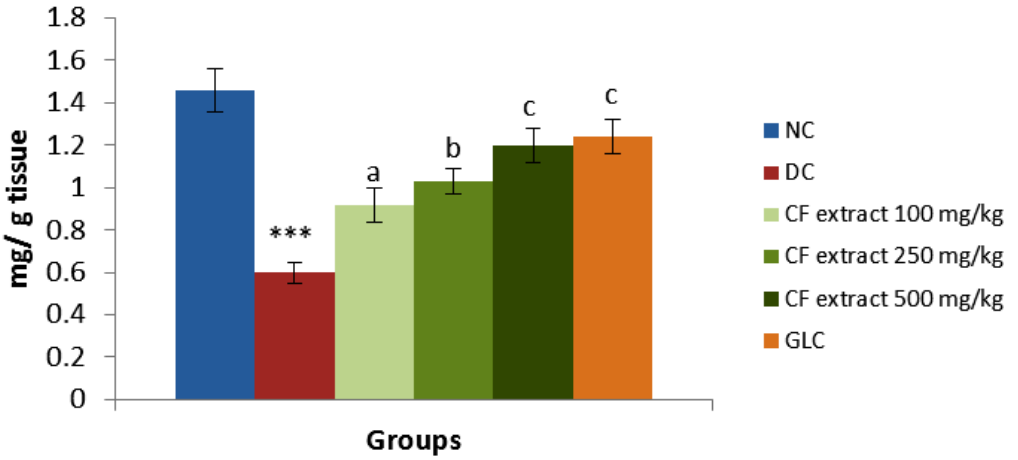

Fig. 6. Effect of extract of *'Cassia fistula'* (CF) on ascorbic acid in testis. ''Significance levels: ***=P≤0.001, comparing diabetic control rats to normal control rats; ns = not significant; a = P≤0.05; b = P≤0.01; c = P≤0.001, comparing *Cassia fistula* extract/glibenclamide-treated diabetic rats to the diabetic control group''

V) resulted in a significant (P≤0.01 and P≤0.001, respectively) rise in the average diameter of seminiferous tubules and Leydig cell nuclei compared to the diabetic group II. Reference drug treated group VI also demonstrated a noteworthy (P≤0.001) increase in the average diameter of STs and Leydig cell nuclei compared to group II.

Diabetic control group II exhibited a noteworthy (P≤0.001) decline in population of spermatogonia, pachytene spermatocytes, preleptotene spermatocytes, likewise round spermatids as relative to normal control group I. There was a dose responsive rise in different types of germ cells, a non-significant increase in the low dose group III (except for round spermatids), a moderately notable (P≤0.001) increase in the medium dose group IV, and a highly significant (P≤0.001) increase in the highest dose group V compared to diabetic control group II. Glibenclamide-treated group VI also exhibited

Table 2. Effects of extract of '*Cassia fistula* ' on seminiferous tubules diameter, nuclear diameter of Leydig cell and histomorphometric indices

Groups	Diameter of STs (μ m)	Nuclear diameter of Leydig cells (μ m)	Spermatogonia	Spermatocyte (Preleptolene)	Spermatocyte (Pachytene)	Spermatid
Group I (Normal Control)	272.67±8.32	6.67±0.33	6.33±0.25	20.17±1.08	23.67±1.52	64.87±2.37
Group II (Diabetic Control)	168.50±9.75***	3.50±0.26***	3.83±0.31***	11.50±0.76***	13.83±1.25***	28.00±2.06***
Group III (Diabetic+100mg/kg of Extract)	200.83±10.15 ns	4.65±0.27 ns	4.85±0.34 ns	14.13± 0.97 ns	17.70±0.95 ns	39.37±2.19 a
Group IV (Diabetic+250mg/kg of Extract)	223.67±9.69 b	5.27±0.21 b	5.52±0.32 b	16.20±0.91 b	19.55±0.90 b	43.97±2.50 b
Group V (Diabetic+500mg/kg of Extract)	242.50±8.30 c	6.10±0.32 c	5.80±0.25 c	17.78±0.61 c	21.60±0.69 b	52.90±3.06 c
Group VI (Diabetic+5mg/kg of Glibenclamide)	248.50±8.69 c	6.20±0.37 c	5.85±0.35 c	17.85±1.01 c	22.00±1.15 c	56.40±3.29 c

"The values are presented as mean ± SEM (n=6), with the following significance levels: *** = P≤0.001, comparing diabetic control rats to normal control rats; ns = not significant; a = P≤0.05; b = Pd"0.01; c = P≤0.001, comparing *Cassia fistula* extract/glibenclamide-treated diabetic rats to the diabetic control group" .

a notable (P≤0.001) rise in the numbers of all types of germ cells, relative to the diabetic control group II.

Histopathological study

Histopathological study of testis of normal control rats of group I exhibited normal testicular histoarchitecture with large no. of spermatozoa found in the lumen (Figure 7a). In contrast, testis of diabetic rats of group II showed significantly (P≤0.001) reduced size of STs and the morphology of the seminiferous epithelium was severely impaired. Many tubules did not contain sperm cells or spermatozoa (Figure 7b). Group III (low dose of *C. fistula* extract) exhibited mild prevention of degenerative changes in the histoarchitecture compared with group II rats (Figure 7c). Administration of medium dose of extract to diabetic rats (group IV) improved testicular histoarchitecture, evidenced by increased number of spermatogenic cells and spermatozoa. Furthermore, there was a significant reduction in the degenerative and vacuolated structure of STs (Figure 7d). The testis of diabetic rats receiving high doses of *C. fistula* extract (group V), exhibited normal histological structure of most STs and an increase of spermatogenic density as the lumen seemed to be filled with sperm (Figure 7e). Standard drug treated diabetic rats (group VI) revealed almost normal appearance of histoarchitecture with a moderate number of spermatozoa (Figure7f).

DISCUSSION

The current study demonstrated diabetes mellitus induced reproductive dysfunctions and highlighted the protective effects of *Cassia fistula* pod extract on several assessed parameters. Notably, there was a marked reduction in absolute weight of testis in STZ-induced diabetic control rats. These findings are compatible with many earlier reports in which similar fall of absolute weight of testis have been reported in STZ-induced diabetic rats.[37,38,39] The decrease in testicular weight in diabetic rats might be attributed to the suppression of testosterone synthesis by Leydig cells as well as loss of spermatids, spermatozoa and Sertoli cells.[40,41] However, diabetic rats receiving treatment with either *Cassia fistula* extract or glibenclamide exhibited a noteworthy

improvement in testis weight. This could be due to better glycemic homeostasis and redox state resulting in restoration of spermatogenic and steroidogenic activities.

The results of the present study indicate that the administration of streptozotocin (STZ) led to a significant reduction in the amounts of insulin, FSH, LH, and testosterone in their serum.

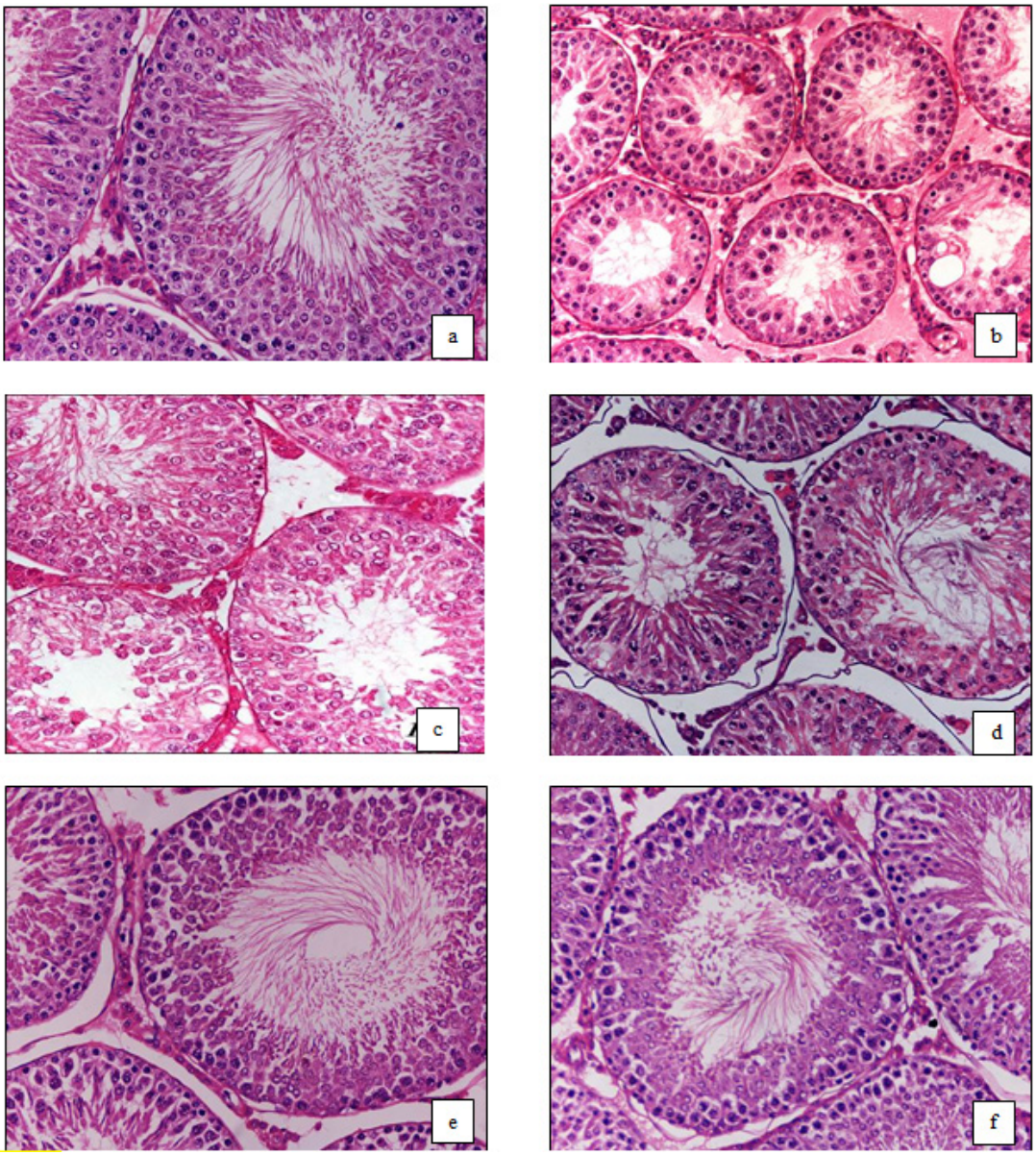

Fig. 7. (a). Photomicrograph of testis from normal control rats displaying typical histoarchitecture. (b). Photomicrograph of testis from diabetic rats showing degenerative and atrophic alterations, as well as disrupted spermatogenesis. (c). Photomicrograph of testis from *C. fistula* extract treated diabetic rats (100 mg/kg b.wt.) showing mild histoarchitectural improvement. (d). Photomicrograph of testis from diabetic rat treated with extract of *C. fistula* (250 mg/kg b.wt.) shows moderate restoration of histoarchitecture. (e). Photomicrograph of testis from diabetic rat treated with extract of *C. fistula* (500 mg/kg b.wt.) showing significant histoarchitectural restoration. (f). Photomicrograph of testis from a glibenclamide-treated diabetic rat displaying nearly normal seminiferous tubule appearance and improved Leydig cell morphology (H & E x200).

These findings are consistent with the observations of several researchers who have documented substantial reductions in insulin levels, concomitant with decreases in testosterone, FSH, and LH levels in STZ-induced diabetic rats.[42,43] Additionally, similar significant decreases in testosterone, FSH, and LH levels have been reported in the diabetic patients by various studies.[44,45]

The reduction in FSH as well as LH observed in the diabetic rats might be linked to deficiency of insulin, as several studies have shown a relationship between levels of insulin and the biosynthesis and secretion of pituitary's LH and FSH hormones.[46,47,48] The decline in serum testosterone level in STZ-induced diabetic rats could be attributed to reduced levels of LH which may cause both, a reduction of total number of Leydig cells and impairment of function of Leydig cell resulting in decrease of biosynthesis and secretion of androgens.[49,50] Furthermore, increased oxidative stress during diabetic state may also cause impairment of steroidogenesis in Leydig cells.[51]

The findings of the present research also demonstrated that treating diabetic rats by *Cassia fistula* extract or glibenclamide led to a notable enhancement in the serum insulin, testosterone, LH, and FSH levels. These outcomes are aligned with previous reports that demonstrated a noteworthy increase in insulin level in experimental diabetic rats following treatment with the methanolic extract of various parts of the *Cassia fistula*[52] or ethanolic extract of leaves[53] or hexane extract of bark[54] or ethanolic extract of flowers[55] of *Cassia fistula*. The observed improvements in LH, testosterone, and FSH levels in serum of diabetic rats treated with *Cassia fistula* extracts could be linked to the rejuvenating effects of the phytoconstituents of the extract. This may lead to improved synthesis and secretion of insulin, consequently facilitating the resumption of Sertoli and Leydig cells functions in testis.[56]

Comparable ameliorative effects of glibenclamide on sex hormones have also been documented in diabetic rats.[57,58] The useful effects of glibenclamide may be due to their insulinogenic action. Glibenclamide blocks the ATP-sensitive potassium (KATP) channels on the β-cell membrane, causing depolarization, activation of the voltage gated Ca^{++} and thereby insulin release.[59]

The study results also exhibited a significant rise in lipid peroxidation (TBARS) and a notable decrease in antioxidant markers like CAT, GSH, AA and SOD in testicular tissues. These findings are uniform with numerous prior studies that reported comparable reductions in antioxidants and elevated TBARS levels in the testicular tissue of diabetic animals.[43,57,60] Hyperglycemia induced by hypoinsulinemia leads to glucose auto-oxidation, glycation of non-enzymatic proteins, activation of the polyol pathway, and the excessive formation of ROS, resulting in oxidative stress.[13] The observed rise in the oxidative stress in testis may be linked to a decrease in levels of testosterone, which is associated with reduced serum insulin concentration.

Cassia fistula extract treatment exhibited a notable decline in TBARS levels and a simultaneous elevation in antioxidant parameters within the testicular tissues of diabetic rats. These findings indicate the robust antioxidant activity of the phytoconstituents contained in the extract such as catechin, epicatechin, anthraquinone, kaempferol, quercetin dehydrate etc.[28,61] These results align with earlier reports that have also indicated improvements in antioxidant parameters alongside a decline in TBARS level in diabetic rats following extracts treatment derived from various parts of *Cassia fistula.*[25,62]

Histological picture of the testis in diabetic control rats revealed marked degenerative and atrophic changes in the testis. There was a marked reduction in the quantity of various types of germ cells and spermatozoa within the STs. The interstitial space was widened and the diameter of STs was also decreased. These findings align with prior research that observed similar atrophic and degenerative changes, along with impaired spermatogenesis, in diabetic rats testes.[37,63,64]

The degenerative and atrophic changes showed in the diabetic rats testes might be linked to the dysfunction of the hypothalamic-pituitary-gonadal (HPG) axis, leading to reduced levels of FSH, LH and testosterone due to insulin deficiency. It has been postulated that LH-mediated proliferation of Leydig cells involves a mechanism incorporating insulin as well as insulin-like growth factor (IGF-

1) signaling.[65] Consequently, inadequate insulin in STZ-induced diabetic rats causes a decline in both the number and metabolic functions of Leydig cells, ultimately contributing to a decline in testosterone levels. The observed reduced nuclear diameter and atrophy of Leydig cells in the present study support this mechanism. Adequate levels of testosterone in the testes are crucial for normal spermatogenesis and the maintenance of the normal morphology of STs.[66] The depletion in different types of germ cell populations within the testes of diabetic rats provides additional evidence for the reduced availability of testosterone. Additionally, degenerative and atrophic changes in Sertoli cells adversely affect spermatogenic functions. Sertoli cells play a vital role in reproductive functions and spermatogenesis, secreting diverse growth factors and transport proteins.[64,67]

Moreover, persistent hyperglycemia and insulin deficiency can cause testicular cells to undergo metabolic adaptations and resort to alternative substrates, which induce oxidative stress.[68] The overproduction of ROS and free radicals can suppress endogenous antioxidant enzymes, disrupt testicular function, and result in apoptosis of germ cells and cell death. Numerous studies have previously observed an increased occurrence of the induction of apoptosis in germ cells within the testes of type 1 diabetic rats and mice.[64,69,70]

Administration of *Cassia fistula* extract/ glibenclamide to diabetic rats led to significant improvements in the histological architecture of their testes. STs showed enlargement, with active spermatogenesis and Leydig cells exhibited morphology close to normal. These beneficial outcomes are likely due to the restoration of testosterone and insulin levels, along with the alleviation of oxidative damage, achieved through the combined action of phytoconstituents present in the extract. Numerous plants with antihyperglycemic properties have demonstrated a mitigating effect on reproductive dysfunction, attributable to the presence of bioactive polyphenolic phytoconstituents.[19,71] Treatment with glibenclamide in diabetic rats also significantly improved testicular histomorphology, consistent with previous findings in diabetic rats.[57,60,72,73]

CONCLUSION

The finding from the current study showed that hydroalcoholic extract of *Cassia fistula* pod significantly ameliorate diabetes induced testicular dysfunctions via multiple mechanism including increase insulin secretion, inhibition of lipid peroxidation, enhancement of the antioxidant activity/concentration and preservation of the concentration of hormone FSH, testosterone and LH. This research provides scientific proof for the traditional utilization of pod of *Cassia fistula* in the control of diabetes mellitus. Additional research is required to identify the particular bioactive compounds responsible for this therapeutic effect.

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The author(s) do not have any conflict of interest.

Data Availability Statement

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Ethics Statement

The animals were maintained in accordance with the guidelines of the CPCSEA and experimental protocol was approved by Institutional animal ethical committee, Department of zoology, University of Rajasthan, Jaipur.

Informed Consent Statement

The informed consent was obtained for experimentation and that it conforms to the standards currently applied in the country of origin. The privacy rights of human subjects must always be observed.

Clinical Trial Registration

This research does not involve any clinical trials.

Permission to reproduce material from other sources

Not applicable

Author Contributions

Ram Niwas Jangir: Data Collection, Methodology, and Writing – Original Draft; Gyan Chand Jain: Conceptualization, Analysis, Supervision & Editing; Aditya Jain: Review editing, Table and figure formatting; Tanvi Jangid: Visualization, Review and editing; Each author mentioned has significantly and directly contributed intellectually to the project and has given their approval for its publication.

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