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Original Article

Protective Effects of *Cassia fistula* Linn. On Reproductive Organs Weight and Tissue Biochemical Markers in Streptozotocin-Induced Diabetic Male Rats

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ABSTRACT

Objectives: This study aims to evaluate the impact of hydroethanolic extract from *Cassia fistula* pods on reproductive organs weight and biochemical markers within reproductive tissues in experimental diabetic rats. **Materials and Methods:** Type 1 diabetes mellitus was induced in male Wistar rats by administering a single intraperitoneal injection of streptozotocin. Diabetic rats were then given oral doses of *Cassia fistula* Pods Extract (CFPE) at 100, 250, and 500 mg/kg BW per day over a period of 60 days. The findings were compared to diabetic rats treated with the standard antidiabetic drug, glibenclamide (5 mg/kg). Measurements included relative weights of the testes, epididymis, vasa deferens, seminal vesicles, and ventral prostate. Additionally, levels of protein, glycogen, cholesterol, sialic acid, acid and alkaline phosphatases in the testes and epididymis, along with fructose content in the seminal vesicles, were analyzed. **Results:** The administration of varying doses of *Cassia fistula* Pod Extract (CFPE) in diabetic rats resulted in a dose-dependent improvement in the relative weights of the testes, epididymis, vasa deferens, seminal vesicles, and ventral prostate. Additionally, CFPE treatment restored the concentrations of protein, glycogen, cholesterol, sialic acid, acid phosphatases, and alkaline phosphatases in the testes and epididymis, compared to the diabetic control group. These findings were comparable with reference drug. **Conclusion:** The results of this study indicate that CFPE effectively improved the reproductive organ weights and restore the tissue biochemical parameters in diabetic male rats.

Keywords: *Cassia fistula*, Glibenclamide, Reproductive organs weight, Streptozotocin, Tissue biochemistry.

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INTRODUCTION

Diabetes Mellitus (DM) is a common and serious metabolic condition marked by consistently high blood glucose levels and major disturbances in the metabolism of carbohydrates, fats, and proteins. It is one of the fastest-growing health conditions globally. According to the International Diabetes Federation (IDF) in 2021, approximately 536.6 million people, accounting for 10.5% of the global population, were living with diabetes. This figure is projected to increase to 738.2 million, representing 12.2% of the world's population, by 2045.[1,2]

Numerous studies have demonstrated that diabetes negatively affects male reproductive function, contributing to male infertility. Experimental research on rodent models has shown that diabetes mellitus is associated with reduced testicular weight, depletion of germ cells and degenerative changes in the testes.[3-6] Studies involving STZ-induced diabetic rats have also reported that diabetes leads to regression and atrophic changes in the epididymis, resulting in a decline in epididymal weight.[3,5,6-8] Additional research has shown that diabetes in experimental rodents causes severe structural and secretory dysfunction, along with reduced weight of accessory sex organs.[3,5,8-12] It is well known that hyperglycemia generates excess of Reactive Oxygen Species (ROS) and attenuates antioxidative defense mechanism.[13] Increasing evidences have indicated that enhancement of oxidative stress play a significant role in pathogenesis of diabetic complications.[14]

Currently available treatment of diabetes including insulin and various oral antidiabetic drugs associated with adverse effects viz. limited efficacy, episodes of hypoglycemia, gastrointestinal intolerance and myocardial infarction etc.,[15-17] Furthermore, many medicinal plants have been reported to restore male reproductive functions in experimental diabetic animals.[18,19]

Cassia fistula Linn. (Family-Caesalpiniaceae) is an indigenous medicinal plant and cultivated throughout India as an ornamental

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tree. In traditional medicine, nearly every part of *Cassia fistula* is used to treat a variety of ailments. The pods of plant are used as abortifacient, anodyne, anti-bilious, antidiabetic, anti-inflammatory, antipyretic, astringent, depurative, diuretic, emollient, purgative and tonic. Phytochemical research has revealed that *Cassia fistula* pods are a rich source of essential minerals such as Potassium (K), Calcium (Ca), Iron (Fe), and Manganese (Mn). The pods of *Cassia fistula* are known to contain various phytochemicals, such as rhein, quercetin dehydrate, kaempferol, dihydrokaempferol, dimeric proanthocyanidin CFI, (-) epiafzelechin, (+) catechin, and 1,8-dihydroxy-3-methylanthraquinone.[20-22]

The hypoglycemic properties of *Cassia fistula* pod extract have been observed in diabetic rats treated with streptozotocin.[23-26] Furthermore, *in vitro* and/or animal model studies have also reported the antioxidant activity of *C. fistula* pods/fruit pulp extract.[27,28]

Despite the promising findings from previous studies, the impact of *Cassia fistula* on diabetes-induced alterations in the weight of male reproductive organs and associated biochemical parameters has not been adequately explored. This study aimed to investigate the effects of the hydroalcoholic extract of *Cassia fistula* (CFPE) on the relative weights of reproductive organs, as well as on the concentrations of protein, glycogen, cholesterol, sialic acid, acid phosphatases, and alkaline phosphatases in the testes and epididymis of STZ-induced diabetic rats. By assessing CFPE's influence on both the weight of reproductive organs and key biochemical markers, this research offers new insights into its potential therapeutic role in restoring male reproductive health under diabetic conditions.

MATERIALS AND METHODS

Preparation Extract

The plant pods were collected and verified by the herbarium curator at the Department of Botany, University of Rajasthan, Jaipur (RUBL21057). Following collection, the pods were rinsed with distilled water and left to air-dry in the shade at room temperature. Once dry, the pods were ground into a coarse powder using an electric grinder. The powdered pods were then suspended in 70% ethanol and left at room temperature for 24 hr. Extraction was performed using a Soxhlet apparatus at a temperature of 60°C-70°C. The extract was filtered, the filtrate was dried in an oven at 40°C to yield a residue, which was stored in an airtight container in a refrigerator until needed.

Animals

Male Wistar albino rats (*Rattus norvegicus*), age 8-10 weeks and weighing 170-210 g, were selected for this study. The rats were housed in standard plastic cages in groups under controlled laboratory conditions, with a maintained temperature of 25±3°C and a 12 hr light-dark cycle in the animal facility of the department. They had unrestricted access to water and a standard pellet diet. The study received ethical clearance from the Institutional Animal Ethical Committee, Department of Zoology, University of Rajasthan, Jaipur, India.

Experimental Induction of Diabetes

Type 1 Diabetes Mellitus (DM) was induced in male rats that had been fasted overnight by administering an intraperitoneal injection of streptozotocin (sourced from Himedia, India), dissolved in citrate buffer (pH 4.5), at a dose of 60 mg per kg of body weight. One week after the injection, fasting blood glucose levels were measured using tail vein blood samples to confirm diabetes. Glucose levels were assessed with a OneTouch glucometer (Johnson and Johnson, USA), and rats with fasting blood glucose levels exceeding 250 mg/dL were classified as diabetic and included in the study.

Experimental Design

The rats were divided into 6 experimental groups (each containing 6 rats) as follows:

Normal Control rats (Group I) were given 0.5 mL DW/day/rat orally for 60 days.

Diabetic Control rats (Group II) were given 0.5 mL DW/day/rat orally for 60 days.

Extract treated diabetic rats (Groups III, IV, and V): Rats received varying doses of CFPE (100, 250, and 500 mg/kg BW per day, respectively), orally for 60 days.

Reference control (Group VI): Rats were administered 5 mg/kg BW of Glibenclamide, orally for 60 days.

24 hr after the final treatment, weight of all rats of various groups were measured and euthanized under mild ether anesthesia for autopsy following an overnight fast.

Organ Weight

The reproductive organs, including the testes, epididymides, vas deferens, seminal vesicles, and ventral prostate, were carefully dissected, cleaned of blood clots and excess fat, and weighed individually using a digital balance. The tissues were then stored at -20°C for later biochemical analysis.

Tissue Biochemistry

Frozen or fresh tissue samples from the testes and epididymis were utilized for quantitative analysis of total protein,[29] glycogen,[30] sialic acid,[31] total cholesterol,[32] acid phosphatase and alkaline phosphatase (Diagnostic kit, Accurex Biomedical Pvt. Ltd., Mumbai, India) and Seminal vesicle tissue used for fructose estimation.[33]

Statistical Analysis

The results are presented as mean±SEM and were evaluated for variance. Data analysis was performed using SPSS version 20.0. Statistical significance was determined using one-way ANOVA, followed by Tukey's *post hoc* test. A *p*-value of less than 0.05 was considered statistically significant.

RESULTS

Reproductive Organ Weight

Changes in the relative weight of reproductive organs of the various experimental groups are presented in Figures 1-5. The relative weight has been expressed as mg/100 g b.wt.±SEM.

Diabetic control rats exhibited a significant reduction ($p \leq 0.01$) in the relative weight of the testes, epididymides, and vas deferens compared to healthy control rats. Administering CFPE at 500 mg/kg BW dose to diabetic rats led to a marked increase ($p \leq 0.05$) in the relative weights of these organs compared to the diabetic control group. However, no significant changes were observed in the low-dose and medium-dose rats. Diabetic rats treated with reference drug also revealed a remarkable increase ($p \leq 0.05$) in the relative weights of all these organs compared to untreated diabetic rats.

The diabetic control rats exhibited a marked reduction ($p \leq 0.001$) in the relative weight of the seminal vesicle compared to the normal control group. Streptozotocin induced diabetic rats treated

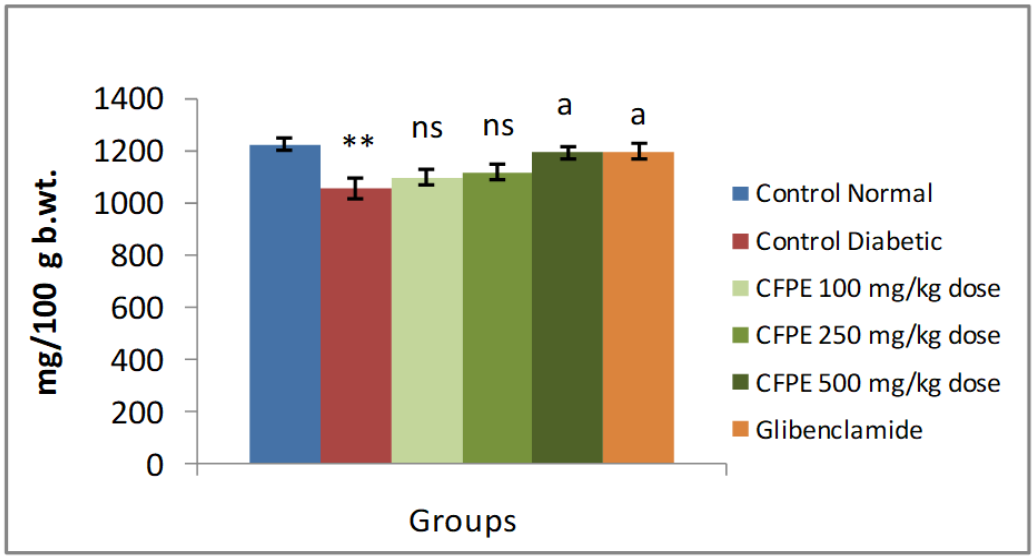

Figure 1: Effects of CFPE on relative weight of testis."Significance levels: **=$p \leq 0.01$, comparing diabetic control rats to normal control rats; ns=non-significant; a=$p \leq 0.05$, comparing CFPE/glibenclamide-treated diabetic rats to the diabetic control group."

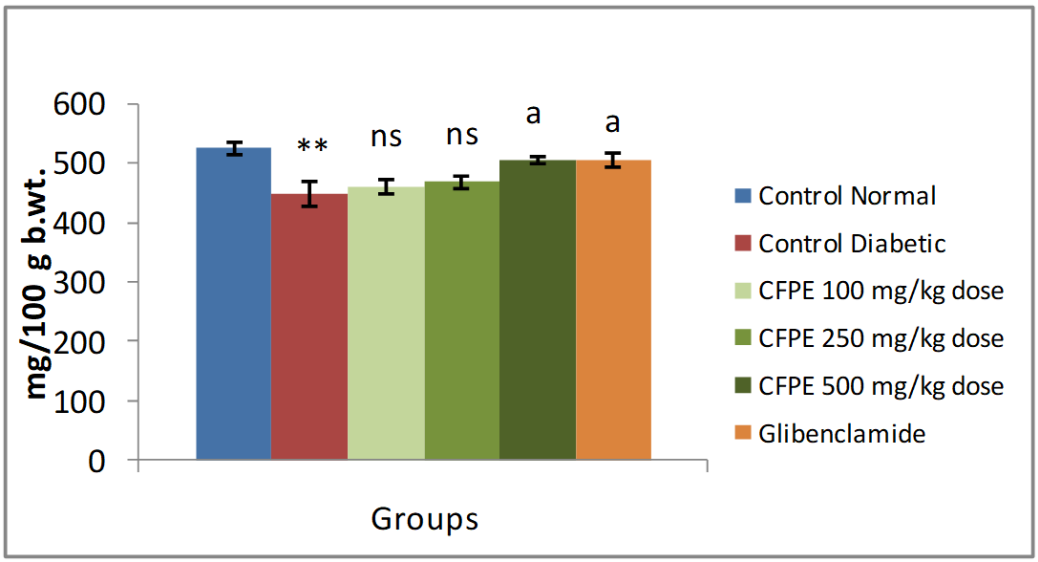

Figure 2: Effects of CFPE on relative weight of epididymis."Significance levels: **=$p \leq 0.01$, comparing diabetic control rats to normal control rats; ns=non-significant; a=$p \leq 0.05$, comparing CFPE/glibenclamide-treated diabetic rats to the diabetic control group."

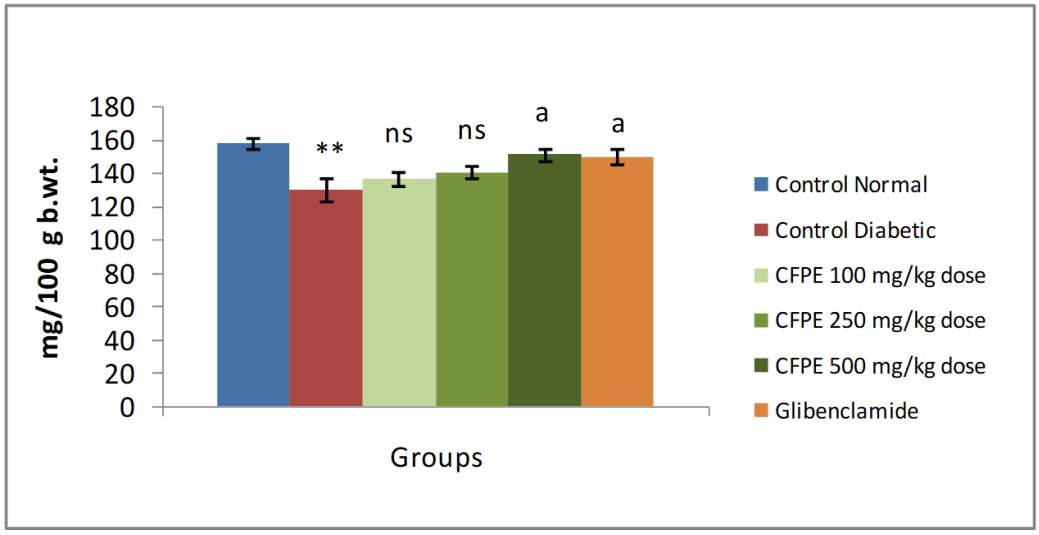

Figure 3: Effects of CFPE on relative weight of vas deferens. "Significance levels: **=$p \leq 0.01$, comparing diabetic control rats to normal control rats; ns = non-significant; a = $p \leq 0.05$, comparing CFPE/glibenclamide-treated diabetic rats to the diabetic control group."

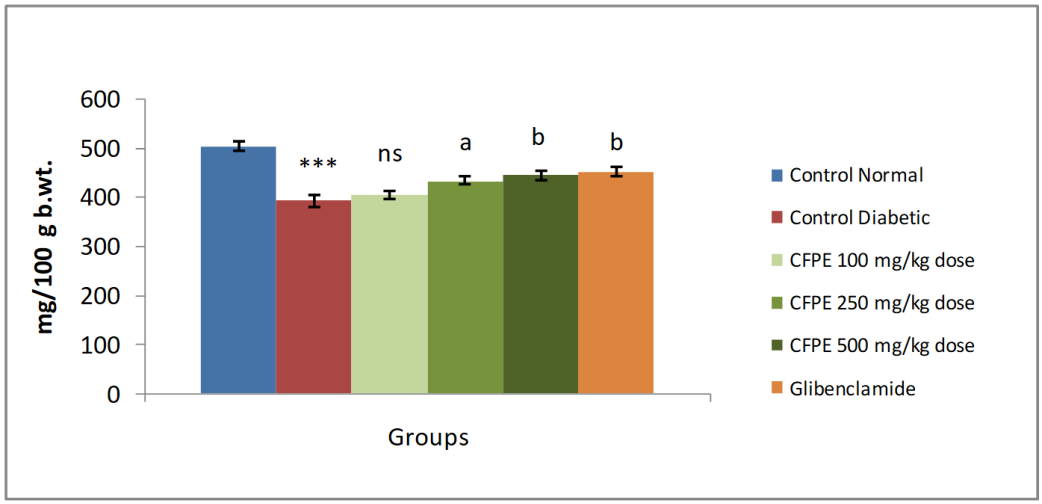

Figure 4: Effects of CFPE on relative weight of seminal vesicle."Significance levels: ***=$p \leq 0.001$, comparing diabetic control rats to normal control rats; ns = non-significant; a = $p \leq 0.05$; b = $p \leq 0.01$, comparing CFPE/glibenclamide-treated diabetic rats to the diabetic control group."

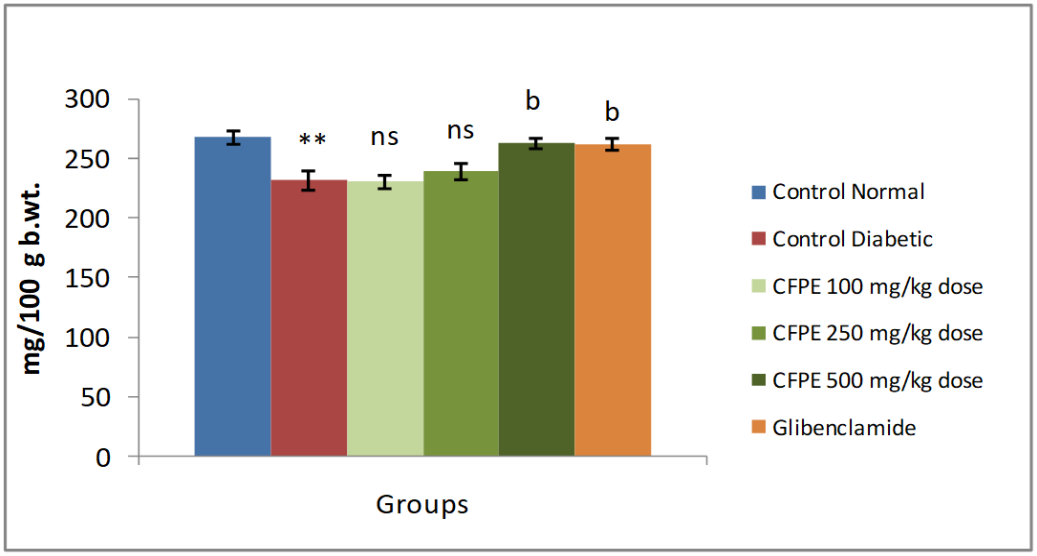

Figure 5: Effects of CFPE on relative weight of ventral prostate."Significance levels: **=$p \leq 0.01$, comparing diabetic control rats to normal control rats; ns = non-significant; b = $p \leq 0.01$, comparing CFPE/glibenclamide-treated diabetic rats to the diabetic control group."

Table 1: Effect of CFPE on Reproductive Tissue Biochemical Markers in STZ-induced Diabetic Rats.

Treatment	Protein (mg/gm)		Glycogen (mg/gm)		Cholesterol (mg/gm)		Sialic acid (mg/gm)		Acid phosphatase (IU/g tissue)		Alkaline phosphatase (IU/g tissue)		Frutose (mg/gm)
	T	E	T	E	T	E	T	E	T	E	T	E	SV
Group I Normal Control	202.96 ±6.83	218.50 ±8.70	3.54 ±0.13	3.34 ±0.19	6.44 ±0.33	6.03 ±0.23	4.95 ±0.18	5.60 ±0.40	2.94±0.15	2.80±0.14	31.83 ±1.61	27.30 ±1.81	5.85 ±0.26
Group II Diabetic Control	139.17± 5.53***	156.33 ±5.92***	2.45 ±0.15***	2.10 ±0.13***	9.28 ±0.30***	8.65 ±0.23***	3.05 ±0.20***	3.22 ±0.16***	2.08 ±0.13**	1.92 ±0.11**	16.26 ±1.02***	13.32 ±.94***	3.85 ±0.27***
Group III Diabetic+CFPE (100mg/kg b.wt.)	156.67 ±6.32^{ns}	178.50 ±7.74 ^{ns}	2.72 ±0.16 ^{ns}	2.33 ±0.11 ^{ns}	8.10 ±0.25 ^{a}	7.93 ±0.36 ^{ns}	3.25 ±0.22 ^{ns}	3.98 ±0.14 ^{a}	2.29 ±0.10 ^{ns}	2.10 ±0.15 ^{ns}	20.18 ±1.14^{ns}	16.78 ±1.52 ^{ns}	4.42 ±0.25 ^{ns}
Group IV Diabetic+ CFPE (250mg/kg b.wt.)	173.67 ±5.64 ^{b}	193.17 ±6.95 ^{b}	3.06 ±0.15 ^{a}	2.75 ±0.12 ^{a}	7.64 ±0.24 ^{b}	7.43 ±0.20 ^{a}	4.04 ±0.17 ^{b}	4.28 ±0.16 ^{b}	2.62 ±0.13 ^{a}	2.43 ±0.13 ^{ns}	23.61 ±1.58 ^{a}	19.95 ±1.39 ^{a}	4.95 ±0.20 ^{a}
Group V Diabetic+ CFPE (500mg/kg b.wt.)	184.83 ±6.96 ^{c}	204.50 ±6.37 ^{c}	3.22 ±0.13 ^{b}	3.08 ±0.24 ^{c}	7.13 ±0.21 ^{c}	6.63 ±0.34^{c}	4.51 ±0.18 ^{c}	4.80 ±0.14 ^{c}	2.76 ±0.12 ^{b}	2.64 ±0.11 ^{b}	25.20 ±1.66 ^{b}	22.11 ±1.44 ^{b}	5.31 ±0.28 ^{b}
Group VI Diabetic+ Glibenclamide (5mg/kg b.wt.)	186.33 ±7.35 ^{c}	208.83 ±6.61 ^{c}	3.26 ±0.10 ^{b}	3.12 ±0.12 ^{c}	7.15 ±0.28 ^{c}	6.56 ±0.32 ^{c}	4.45 ±0.18 ^{c}	5.02 ±0.24 ^{c}	2.82 ±0.09 ^{b}	2.68 ±0.15 ^{b}	27.25 ±1.33 ^{c}	23.46 ±1.68 ^{c}	5.40 ±0.24 ^{b}

"Values represent mean±SEM (n=6), Level of significance: ** = $p\leq0.01$; *** = $p\leq0.001$, diabetic control rats compared with normal control rats. ns= non-significant; a = $p\leq0.05$; b = $p\leq0.01$; c = $p\leq0.001$, *C. fistula* extract or glibenclamide treated rats compared with diabetic control rats"

with a medium dose ($p\leq0.05$) or a high dose ($p\leq0.01$) of CFPE, as well as those treated with glibenclamide ($p\leq0.01$), showed a significant increase in the relative weight of the seminal vesicle compared to diabetic control. However, the low-dose treatment group exhibited a non-significant increase in the relative weight of the seminal vesicle.

The relative weight of the ventral prostate was significantly reduced ($p\leq0.01$) in the diabetic control group compared to the normal control group. Treatment with the high dose (500 mg/kg b.wt.) of CFPE or glibenclamide led to a significant ($p\leq0.01$) increase in ventral prostate weight compared to the diabetic control group. In contrast, the administration of low (100 mg/kg b.wt.) and medium (250 mg/kg b.wt.) doses of CFPE resulted in a non-significant increase in ventral prostate weight in the diabetic rats.

Tissue Biochemistry

Table 1 presents the results of biochemical parameter analyses across various tissue samples in the different experimental groups.

The protein content in the testis and epididymis was significantly lower in diabetic rats compared to normal control rats. Treatment with CFPE in diabetic rats at doses of 250 mg/kg BW ($p\leq0.01$) and 500 mg/kg BW ($p\leq0.001$) resulted in a significant increase in the protein content of both the testis and epididymis compared to diabetic control rats. However, in the low-dose (100 mg/kg BW) group, the increase in protein content in the epididymis and testis was not significant. Diabetic rats treated with the reference drug also showed a remarkable ($p\leq0.001$) rise in the total protein content of both the testis and epididymis compared to untreated diabetic rats.

In STZ-induced diabetic rats, Glycogen concentration in both the epididymis and testis were significantly lower ($p\leq0.001$) compared to normal control rats. Glycogen concentration in the testis was notably higher ($p\leq0.05$ and $p\leq0.01$) in diabetic rats treated with medium or high doses of CFPE compared to the untreated diabetic group. No significant change in testicular glycogen concentration was observed in the low-dose group. Diabetic rats treated with reference drug also exhibited a significant ($p\leq0.01$) increase in testicular glycogen content compared to untreated diabetic rats. Likewise, in the epididymis, glycogen concentration was significantly higher ($p\leq0.05$ and $p\leq0.001$) in diabetic rats treated with medium and high doses of CFPE as well as reference drug treatment also caused a highly significant ($p\leq0.001$) increase in epididymal glycogen concentration compared to untreated diabetic rats.

In STZ-induced diabetic rats, cholesterol levels in both the epididymis and testis were significantly higher ($p\leq0.001$) compared to normal control rats. However, in diabetic rats treated with three different doses of CFPE (100, 250, and 500 mg/kg BW), the elevated cholesterol levels in the testis showed a dose-dependent reduction (P≤0.05, P≤0.01, and $p\leq0.001$) in comparison to the untreated diabetic group. Cholesterol levels in the epididymis

also decreased in a dose-dependent manner in CFPE-treated rats, with a non-significant reduction in the low-dose group, a slight reduction ($p \leq 0.05$) in the medium-dose group, and a highly significant reduction ($p \leq 0.001$) in the high-dose group compared to untreated diabetic rats. Glibenclamide treatment also significantly ($p \leq 0.001$) prevented the increase in cholesterol levels in both the epididymis and testis in diabetic rats.

STZ administration in rats caused a highly notable ($p \leq 0.001$) reduction in sialic acid levels in both the epididymis and testis in comparison to the normal control rats. Diabetic rats treated with CFPE at doses of 250 and 500 mg/kg BW/day revealed a considerable ($p \leq 0.01$ and $p \leq 0.001$) increase in sialic acid levels in both the epididymis and testis compared to diabetic control rats. In the low-dose group (100 mg/kg BW), a mild but evident ($p \leq 0.05$) increase in sialic acid concentration was observed only in the epididymis. Diabetic rats treated with glibenclamide also revealed a marked ($p \leq 0.001$) increase in sialic acid levels in both the testis and epididymis compared to untreated diabetic rats.

Compared to normal control rats, STZ-induced diabetic rats showed a notable ($p \leq 0.01$) reduction in acid phosphatase activity in both the testis and epididymis. Diabetic rats treated with CFPE (500 mg/kg BW) or glibenclamide showed a considerable ($p \leq 0.01$) rise in acid phosphatase activity in both the testis and epididymis. Diabetic rats receiving the medium dose (250 mg/kg BW) of CFPE showed a slight but evident ($p \leq 0.05$) increase in acid phosphatase activity in the testis only. However, in the low-dose group, acid phosphatase levels remained unchanged in both the testis and epididymis.

A significant ($p \leq 0.001$) reduction in alkaline phosphatase activity in both the epididymis and testis was observed in diabetic control rats compared to normal control rats. However, administration of CFPE at medium (250 mg/kg BW) and high (500 mg/kg BW) doses resulted in significant ($p \leq 0.05$ and $p \leq 0.01$, respectively) increase in alkaline phosphatase activity in both the epididymis and testis of diabetic rats compared to diabetic control rats. In the low-dose group, alkaline phosphatase level in both the testis and epididymis remain unchanged. Glibenclamide treatment also caused a highly significant ($p \leq 0.001$) increase in alkaline phosphatase activity in both the epididymis and testis of diabetic rats compared to untreated diabetic rats.

Streptozotocin administration in rats caused a highly notable ($p \leq 0.001$) reduction in fructose content in the seminal vesicle compared to normal control rats. Diabetic rats treated with CFPE at dose levels of 250 and 500 mg/kg BW showed considerable ($p \leq 0.05$ and $p \leq 0.01$, respectively) increase in fructose concentration in the seminal vesicle compared to untreated diabetic rats. Treatment with glibenclamide also resulted in a marked ($p \leq 0.01$) rise in fructose levels in the seminal vesicle compared to the diabetic control group.

DISCUSSION

This study observed a notable reduction in the relative weight of the epididymis, testis, vas deferens, ventral prostate and seminal vesicles in STZ-induced diabetic control rats. These results align with several previous studies that have reported similar reductions in the weight of the testis and accessory sex organs in experimentally-induced diabetic rats.[34-36] The decline in the weight of the genital organs is likely due to lower serum testosterone levels in the diabetic state, since testosterone is essential for maintaining the structure and function of the testis and accessory sex organs. Insulin deficiency in diabetes leads to a decrease in serum testosterone levels by suppressing the production of LH and FSH.[37,38]

Diabetic rats treated with CFPE showed a significant improvement in the weight of the testis and other accessory reproductive organs. This improvement may be due to better glycemic control and redox balance, which help restore spermatogenic and steroidogenic functions. These results are consistent with several studies that have reported a significant increase in the weight of reproductive organs in diabetic rats treated with extracts from various antihyperglycemic plants.[39-41] Diabetic rats treated with glibenclamide also exhibited positive effects on the weight of the testis and accessory reproductive organs, supporting findings from previous research.[34,42,43]

Protein biosynthesis plays a crucial role in testicular development and spermatogenesis. Sertoli cells produce and release various proteins that are essential during different stages of germ cell maturation. Additionally, spermatogonia and primary spermatocytes, which are responsible for cell renewal, are also highly involved in protein synthesis. Androgens have a significant impact on protein synthesis within the testes.[44] In this study, STZ-induced diabetic control rats showed a significant decrease in testicular protein content compared to control rats. These results are consistent with previous studies that have reported similar reductions in testicular protein levels in diabetic rats.[45-47] This decline in testicular protein concentration may be due to disruptions in testosterone production and testicular secretory activity, disturbances in protein synthesis or metabolism, or a reduction in the number of germ cells and spermatozoa in the testis.[48,49]

The total protein concentration in the epididymis was significantly reduced in diabetic control rats. This reduction in protein levels suggests a deficiency in androgens, a loss of spermatozoa, and decreased secretory activity of the epithelium.[50,51] The lower protein concentration in the epididymis may alter its internal environment, potentially impairing sperm maturation. Treatment of diabetic rats with CFPE or glibenclamide showed significant improvement in total protein concentration in testes and epididymis. This might be due to the restoration of the insulin secretion and glucose homeostasis in the blood as well

as increase in testosterone level and secretary functions of these organs. These findings are consistent with previous studies which also reported significant improvement in the concentrations of testicular protein in diabetic rats treated with antidiabetic plant or glibenclamide.[45-47]

In this study, the glycogen concentration was significantly reduced in both the testis and epididymis of diabetic control rats. These results align with earlier studies that observed a similar reduction in glycogen content in the testis of diabetic rats.[52,53] The reduction in glycogen levels in the epididymis and testis was restored in diabetic rats following treatment with CFPE or glibenclamide. This restoration may be attributed to the improvement in insulin secretion and glucose homeostasis. These results align with earlier studies where significant increase in glycogen content in the testis of diabetic rats were observed after treatment with plant extracts with antidiabetic properties or glibenclamide.[52,53]

This study observed a notable increase in cholesterol content in both the epididymis and testis of diabetic control rats. These results are in line with earlier studies that reported a similar increase in cholesterol levels in the testis of diabetic rats.[34,52] The elevated cholesterol content could be attributed to impaired androgen biosynthesis due to decreased LH secretion, a result of insulin deficiency in the diabetic state. Additionally, reduced testosterone levels and impaired activity of steroidogenic enzymes in Leydig cells have been previously reported in diabetic rats.[36,52]

Administering CFPE or glibenclamide to diabetic rats significantly prevented cholesterol accumulation in the testis and epididymis. This might be due to restoration of steroidogenesis and spermatogenic activity under the influence of phytoconstituents present in the extract. Many other studies have also shown ameliorative effect of plant extracts/ glibenclamide on testicular cholesterol levels in diabetic rats.[34,54]

Sialic acid, secreted by the principal cells of the epididymis and functions as a decapacitation factor, preventing premature capacitation of spermatozoa.[10] In this study, a marked reduction in sialic acid levels was observed in the testis and epididymis of diabetic control rats compared to normal control rats. This reduction may be linked to a deficiency in testosterone associated with diabetic condition. These findings align with earlier research, which also reported a marked reduction in sialic acid concentrations in the testis[46] and epididymis[10] of diabetic rats. Treatment with CFPE or glibenclamide significantly restored sialic acid levels in both the epididymis and testis of diabetic rats, likely due to an increase in serum sex hormone levels and a reduction in oxidative stress.

This study found a marked decrease in Acid Phosphatase (ACP) activity in both epididymis and testis of diabetic control rats compared to normal controls. The decreased ACP activity in the testis may indicate testicular degeneration, likely resulting from impaired testicular steroidogenesis, elevated oxidative stress, and reduced gonadotropin secretion caused by insulin deficiency in the diabetic state. Similarly, the decline in ACP activity in the epididymis can be attributed to a reduction in sperm count and activity. Earlier studies have similarly reported a comparable reduction in ACP activity in the testis of diabetic rats.[55,56] However, contrary findings from some researchers have indicated an increase in testicular ACP activity in diabetic rats,[57] possibly due to differences in the duration of treatment. Treatment with CFPE or glibenclamide effectively restored ACP activity in both epididymis and testis of diabetic rats, likely due to enhanced steroidogenic and gametogenic activity promoted by the phytoconstituents in these extracts.

In the present study diabetic control rats showed a significant decline of the Alkaline Phosphatase (ALP) activity in both epididymis and testis as compared to normal control rats. These results are in accordance with Atta *et al.* (2017)[55] who also observed significant decline in testicular ALP activity in diabetic rats. However, other workers have reported an increase in ALP activity in diabetic rats.[56,58] This discrepancy in results might be due to different duration of the experiment. The decreased alkaline phosphatase activity in the epididymis of diabetic control rats may result from degenerative changes in the epididymal epithelium and a reduced density of spermatozoa in the lumen. Treatment with CFPE or glibenclamide significantly restored alkaline phosphatase activity levels in both the testis and epididymis. This improvement could be attributed to enhanced sex hormone secretion, improved spermatogenic activity, and the alleviation of oxidative stress alongside better insulin secretion.[55]

The fructose concentration in the seminal vesicle was significantly lower in diabetic control rats compared to normal control rats. These findings align with several previous studies that have also reported a marked decrease in fructose levels in the seminal vesicle of diabetic rats. Treatment with CFPE or glibenclamide significantly enhanced fructose concentrations in the seminal vesicle of diabetic rats.[42,52,56,59] The administration of CFPE or glibenclamide to diabetic rats notably improved the fructose levels in the seminal vesicle. This observed increase in fructose content in seminal vesicles might be correlated with improved testosterone level, spermatogenic activity and increased insulin secretion by the bioactive phytoconstituents present in the extracts. These results are further supported by the findings of other researchers who have also reported restoration of fructose level in seminal vesicle of diabetic rats after treatment with glibenclamide and plant extracts having antidiabetic activity.[17,52,56,59]

CONCLUSION

The findings from the present study indicated the ameliorative effect of CFPE on diabetes induced biochemical alteration in testicular and epididymal tissue and improvement in reproductive organs weight. The possible mechanisms of this action might be due to their antidiabetic and antioxidative effects.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICS APPROVAL

The study was carried out under the supervision of ethical committee and the experimental protocol was approved by the departmental animal ethical committee, Department of Zoology, University of Rajasthan, Jaipur and CPCSEA guidelines were followed to maintain the experimental animals.

ABBREVIATIONS

DM: Diabetes Mellitus; **CFPE:** *Cassia fistula* pod extract; **BW:** Body weight; **STZ:** streptozotocin; **SEM:** Standard error of mean; **ALP:** Alkaline phosphatase; **ACP:** Acid phosphatase; **LH:** Luteinizing Hormone; **FSH:** Follicle stimulating Hormone.

SUMMARY

The study investigates the effects of a hydroethanolic extract from *Cassia fistula* pods on reproductive organ weights and biochemical markers in diabetic rats. The rats were induced with Type 1 diabetes and given oral doses of *Cassia fistula* pods extract (CFPE) over 60 days. Results showed that CFPE significantly increased reproductive organ weights and improved biochemical parameters in reproductive tissues, indicating its effectiveness in treating diabetic male rats.

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