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Research Article

Synergistic effect of Camel milk and *Hibiscus rosa sinensis* on the reproduction of Type II diabetic albino Wistar rats

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Abstract
Diabetes adversely affects male reproduction and closely associated with male infertility. The present study designed to assess the synergistic effects of Camel milk and *Hibiscus rosa sinensis* on the reproduction of type II diabetic Wistar rats over one month. Rats were divided into seven groups: control (Group I) and diabetic control (Group II). Group III received 125 mg/kg/day *H. rosa sinensis*, whereas Group IV received 50 ml/rat/day Camel milk. Groups V–VII were treated synergistically with varying H. rosa sinensis and Camel milk dosages. Group II showed persistent hyperglycemic state, gradually decreasing in treatment groups (III-IV) and synergistic groups (V-VII). The pancreas of Group II rats was damaged, including acinar cell necrosis and vacuolation. These damages were significantly improved in treatment groups (III-IV) and synergistic groups (V-VII). Group II male rats exhibited significant damage to their seminiferous tubules, including a decrease in diameter, and loss of spermatozoa. Seminiferous tubule structures were significantly improved in treatment groups III & IV. The 1st synergistic group (V) demonstrated greater recovery of altered seminiferous tubule structures than the 2nd and 3rd synergistic groups (VI and VII). Sperm analysis revealed that Group II animals had significantly reduced sperm count, motility, and viability, whereas treatment groups III-IV had better sperm characteristics. The synergistic group (V) had a greater sperm count, motility, and viability than synergistic groups (VI and VII). The present study showed synergistic benefits of *H. rosa sinensis* and Camel milk in treating and reproducing type II diabetic rats at very low doses.

Keywords: Camel milk, Glycemic level, *Hibiscus rosa sinensis*, Histology, Type II diabetes

INTRODUCTION

Diabetes mellitus is characterized by elevated blood glucose levels and is a chronic metabolic disease reported by the World Health Organization (WHO, 2016). This illness can potentially harm the heart, blood vessels, kidneys, eyes, and nerves over time (Galicia-Garcia *et al*., 2020). Out of the three primary forms of diabetes, type II diabetes accounts for over 90% of all occurrences, making it significantly more common than type 1 diabetes or gestational diabetes (Defronzo *et al*., 2015). Type 2 diabetes is characterized by relative insulin insufficiency resulting from pancreatic β-cell dysfunction and insulin resistance in target organs. Between 1980 and 2004, the incidence and prevalence of type 2 diabetes grew fourfold due to global increases in obesity, sedentary lifestyles, and an aging population (Chatterjee *et al*., 2017). Cardiovascular disease is the main cause of morbidity and mortality associated with type II diabetes. To lower the risk of complications and the condition's progression, tight control over blood pressure, glucose, and cholesterol levels is necessary (Ma*et al*., 2022). Diabetic mellitus can cause long-term damage, dysfunction, and failure of multiple organs, including retinopathy with potential blindness, nephropathy leading to renal failure, peripheral neuropathy with foot ulcer risk, amputation risk, Charcot joints, and autonomic neuropathy causing gastrointestinal, genitourinary, cardiovascular, nd sexual dysfunction. A significant side effect of diabetes disrupts male reproductive system (Bodman *et al*., 2023; Chauhan *et al*., 2023). The most common sexual dysfunction that is recorded is erectile dysfunction, which affects 35 to 75% of males with diabetes. Hypogonadism affects up to 40% of males with type II diabetes and metabolic syndrome. The combined effect of these anomalies could be reduced male fertility (Ray and Pramanik, 2020). Diabetes mellitus lowers testosterone levels, sperm motility,

sperm count, and sperm viability, in addition to causing morphological damage to the testis (Saumya and Basha, 2017).

Many traditional food therapies and natural cures have been available to treat diabetes without side effects. In Africa, Asia, and the Middle East, Camel milk is traditionally believed to help prevent and control diabetes. Additionally, epidemiological research has demonstrated that people who drink camel milk in the same community have a noticeably reduced incidence of diabetes than people who do not (Zheng *et al*., 2021).

Also, well known for its therapeutic properties in medicine *Hibiscus rosa sinensis* belongs to the Malvaceae family and is found in tropical regions. According to reports, the plant has anti-inflammatory, anticancer, antifertility, anti-ovulatory, antiviral, antifungal, antibacterial, hypoglycemic, cardioprotective, and antioxidant qualities. Their leaves, roots, and flower petals are mostly used to make remedies (Pillai and Mini, 2016). Previous research suggests that *H. rosa sinensis* and Camel milk have anti-diabetic qualities. Conversely, little is known about the potential benefits of Camel milk and *H. rosa sinensis* in treating type II diabetes or the reproduction of diabetic rats. More research is needed to investigate these factors adequately. Therefore, this study aimed to assess how camel milk and *H. rosa sinensis* combined to affect the reproduction of type II diabetic albino Wistar rats.

MATERIALS AND METHODS

Chemicals

Streptozotocin (STZ), Nicotinamide (NAD), and Citric acid monohydrate (CAM) were purchased from Sigma Chemicals.

Hibiscus rosa sinensis ethanolic flower extract

Fresh flowers were plucked from the university area and air-dried at room temperature. Following that, their powder was produced, and for 24 hours, 50 g of it was dissolved in 300 ml of ethanol. The extract was then filtered and dried at room temperature (Sankaran and Vadivel, 2011).

Wistar albino rats

Forty-two Wistar albino rats (3–4 months old) weighing 150-200 gm were obtained from the disease-free small animal house (DFSAH) in Hisar, Haryana. They were kept in controlled environments in polypropylene cages with six rats per cage.

Ethical approval

This study was approved by M. D. University's Institutional Animal Ethics Committee (IAEC) (Reg. no. 1767/GO/Re/S/14/CPCSEA), Rohtak with approval number CAH/282-93/11-12-21.

Camel milk

Fresh camel milk was bought daily from a nearby farmer for this study.

Experimental induction of type II diabetes

After intraperitoneal delivery of nicotinamide (NAD) (200 mg/kg) prepared in 0.9% saline and after 15 minutes, rats received a single intraperitoneal injection of 55 mg/kg of STZ, freshly dissolved in cold citrate buffer (pH 4.5). This caused experimental type II diabetes in the rats. The rats' blood glucose levels were assessed after 72 hours to identify the hyperglycemia state. For this investigation, rats with blood glucose levels greater than 220 mg/dL were classified as diabetic (Abdel-Moneim *et al*., 2017).

Experimental design

Seven groups of six rats (N = 6) each were formed as one control group (Group I), and six groups (Group II to VI) of rats with diabetes. The duration of this study was one month.

Group I: Served as a standard control group.

Group II: Served as a control group for diabetics and was given Streptozotocin (STZ) only.

Group III: Diabetic rats received an oral dose of 125 mg/kg of *H. rosa sinensis* flower extract (Sankaran and Vadivel, 2011).

Group IV: Diabetic rats received 50 ml of Camel milk per rat per day (Raj *et al*., 2023).

Group V: It was regarded as synergistic group 1st. In this group, diabetic rats received Camel Milk and *H. rosa sinensis* at doses of 12.5 ml/rat/day and 31.25 mg/kg, respectively.

Group VI: It was regarded as the Synergistic Group 2nd, given doses of *H. rosa sinensis* and Camel milk, respectively, of 62.5 mg/kg and 25 ml/rat/day.

Group VII: It was regarded as Synergistic Group 3rd. In this group, diabetic rats received 50 ml/rat/day and 125 mg/kg of Camel Milk and *H. rosa sinensis*, respectively.

Histopathological analysis

Organs such as the pancreas and testis were removed from the animals after they were dissected, and they were then placed in a 10% formalin solution. Following the tissue preparation for the slide preparation using running tap water, it was washed in xylene and dehydrated using ethanol in increasing concentrations of 30%, 50%, 70%, 90%, and 100% ethanol solution. The block was formed using paraffin wax, and tissue slices measuring 5 µm were stained with hematoxylin and eosin for histological analysis. These sections were examined using a light microscope at 200x magnification (Shuklan *et al*., 2023).

Sperm analysis

Sperm analysis is a test used to evaluate fertility. Rats

underwent anesthesia, and their testicles were extracted carefully. Then, sperm motility, viability, and count were performed by gently removing their cauda epididymis from the testis (Vasan, 2011).

Sperm count

The spermatozoa from the rats were pressed into a petri plate with five milliliters of physiological saline after the cauda epididymis was removed. This liquid was combined with 1 ml of the semen-diluting fluid in 0.5 ml. One drop of this mixture was placed on the hemocytometer. After that, spermatozoa were counted under the light microscope (Srinivasulu and Changamma, 2017).

Sperm motility

At 37 °C, prewarmed normal saline was used to mince the epididymis. To examine 200 motile sperm in four distinct fields, one drop of sperm suspension was put on a glass slide. After the sperm were separated from the epididymis, their motility was assessed under a microscope in a matter of seconds or minutes (Pant and Srivastava, 2003).

Sperm viability

The sperm's viability was assessed using eosin and nigrosin staining. Two drops of 1% eosin Y were combined with one drop of sperm suspension. Three drops of 10% nigrosin were added and well mixed after 30 seconds. On a spotless glass slide, a drop of the mixture was applied, and it was then let to air dry to create a thick smear. A phase contrast microscope was used to analyze the prepared slide. Pink-colored dead sperm were distinguished from live, unstained sperm by counting their numbers (Maiti, 2017).

Oral glucose tolerance test (OGTT)

This test was carried out before the rats were sacrificed. After a glucose solution (2 g/kg) was administered orally, the rats' glucose levels were measured at 0, 30, 60, 90, and 120 minutes. Next, the OGTT was assessed (Meena *et al*., 2016).

Statistical analysis

Graph Pad PRISM version 8.0 was used to statistically analyze the acquired data, and the results were displayed as Mean ± SD. Tukey's post hoc test and two-way analysis of variance (ANOVA) were used to compare the variables of the different experimental groups, with a significance level of $p < 0.05$.

RESULTS

Alterations in glycemic level

The glycemic levels of each animal in each group were monitored every five days throughout the 30-day experimental period, are indicated in Fig. 1. On the first day, the average blood glucose level (136.16 ± 11.66 mg/dL) was only seen in the animals in the Group I. Up to the 15th day of the experiment, glycemic levels of all other experimental groups (Group II to VII) were greater than 250 mg/dL, indicating a significant ($p < 0.01$) increase from the Control group. On the 20th, 25^{th,} and 30th day, the treatment groups (Groups III to VII) showed significantly ($p < 0.0001$) lower glucose levels than the Group II animals.

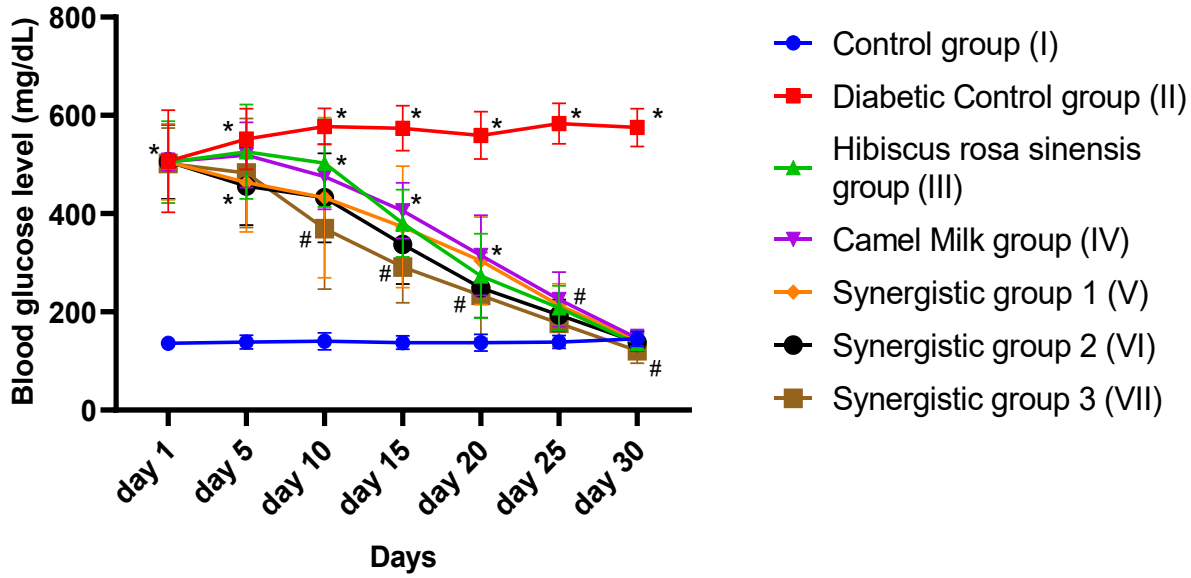

Fig. 1. *Changes in the glycemic level of various groups from Day 1 to Day 30. Data is represented as Mean ± SD, N = 6. *Values are significantly different from Group I at p < 0.01. #Values significantly differ from Group II at p < 0.001*

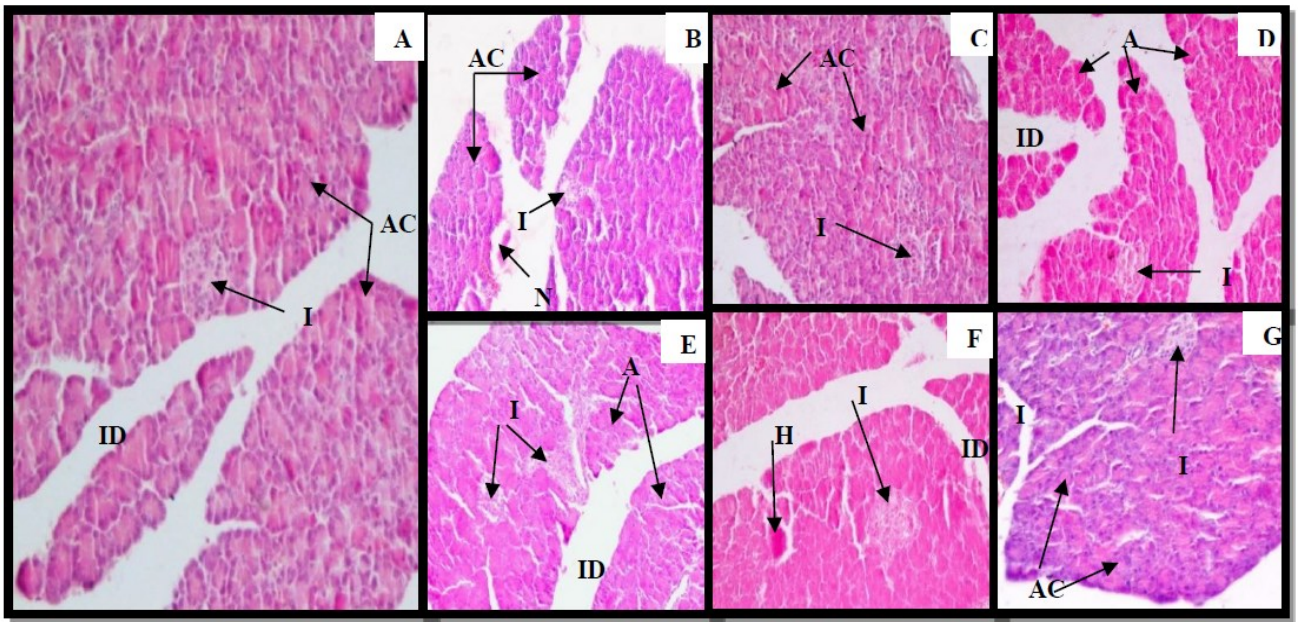

Fig. 2. *Histological analysis of pancreas sections of rats of different groups, stained with Hematoxylin & Eosin (200x). (A) Group I showing the impact structure of pancreatic acinar cells, islet of Langerhans, and intralobular duct. (B) Group II showing damaged pancreatic islets, necrosis of acinar cells, and vacuolation. (C) Group III showing remarkable improvements in the impact structure of islets of Langerhans, acinar cells, and the intralobular duct. (D) Group IV showing the normal structure of islets and acinar cells. (E) Group V showing normal islets and acinar cells but some necrosis was observed. (F) Group VI showing improved islets of Langerhans but some hemorrhage was also observed. (G) Group VII showing the improved structure of the islets of Langerhans. AC, acinar cells; ID, intralobular duct; I, islets of Langerhans; N, necrosis; H, hemorrhage*

On the final day of the trial, the Diabetic control group's blood glucose level (575.16 ± 38.23 mg/dL) was considerably ($p < 0.0001$) higher than the animals in the Control group (145.33 ± 15.13 mg/dL). In comparison to Group II, all treatment groups (III to VII) had remarkably reduced their glycemic levels, which returned to the normal range (< 200 mg/dL) after the experiment.

Histopathological study

Pancreas

The pancreatic architecture of Group I rats was normal and compact, showing a clear intralobular duct, pancreatic islet, and acinar cells (Fig. 2A). Group II rats (Fig. 2B) showed necrosis of pancreatic acinar cells, due to which vacuolation was observed. The size of pancreatic islets was reduced in this group due to some damage caused by STZ. Group IV (Fig. 2D) and Group III (Fig. 2C), all of these damages were reversed, resulting in the pancreas's normal and compact histological structure. Group V (Fig. 2E) showed a normal structure like that of Group I (Fig. 2A) rats. Group VI (Fig. 2F) and Group VII (Fig. 2G) showed signs of improvements. However, some necrosis and hemorrhage were observed in these groups.

Testis

The testicular section of Group I showed a well-ordered structure of seminiferous tubules, spermatogonia, spermatocytes, spermatids, spermatozoa, and Leydig cells (Fig. 3A). Group II was observed to have severely damaged structure of seminiferous tubules with fewer spermatozoa, wide lumen and large interstitial space (Fig. 3B). The diameter was seminiferous tubules was drastically reduced in this group as compared to Control group. Group III (Fig. 3C), Group IV (Fig. 3D), and Group V (Fig. 3E) showed the normal compact structure of seminiferous tubules, spermatogonia, and spermatozoa like that of Group I. Group VI (Fig. 3F) and Group VII (Fig. 3G) showed the damaged structure of seminiferous tubules. In these groups, fewer spermatozoa, wide lumen, large interstitial space, and disruption of intercellular junction between seminiferous tubules were observed.

Sperm analysis

Sperm analysis (Table 1) demonstrated that Group II showed extremely ($p < 0.0001$) lower sperm count, motility, and viability than Group I. Group III demonstrated significantly ($p < 0.0001$) higher sperm count, motility, and viability than Group II. Group IV and Group V had significantly ($p < 0.0001$) higher motility and viability than Group II. Group VI and Group VII showed extremely ($p < 0.0001$) lower sperm count, motility, and viability than Group I.

Oral glucose tolerance test (OGTT)

Following a 30-day treatment, the OGTT responses varied throughout the groups (Fig. 4). Group II demon-

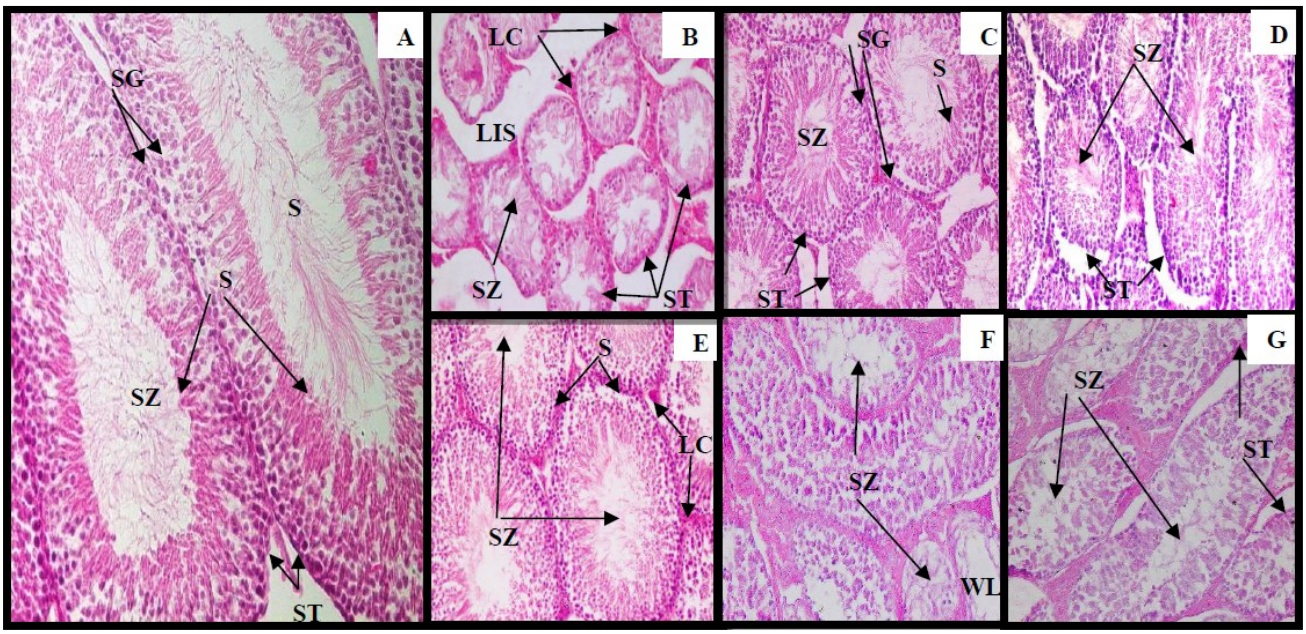

Fig. 3. *Histological analysis of testicular sections of rats of different groups of Type II diabetes, stained with Hematoxylin & Eosin (200x). (A) Group I showing the normal structure of seminiferous tubules, spermatogonia, spermatids, and spermatozoa. (B) Group II showing large intercellular space between seminiferous tubules, wide lumen, loss of spermatozoa, etc. (C) Group III showing the normal structure of seminiferous tubules. (D) Group IV also showing the normal structure of seminiferous tubules. (E) Group V also showing the well-improved structure of seminiferous tubules with normal spermatogonia, spermatids, and spermatozoa. (F) Group VI showing the damaged structure of seminiferous tubules with a wide lumen. (G) Group VII showing large intercellular spaces, few spermatozoa, and damaged spermatocytes. ST, seminiferous tubules; LC, Leydig cells; SG, spermatogonia; S, spermatids; SZ, spermatozoa; LIS, large intercellular space; WL, wide lumen*

strated a lower tolerance to oral glucose than Group I, as evidenced by glycemic levels of 575 ± 31.89, 595 ± 10, 598.75 ± 2.50, 554.50 ± 12.23, and 562 ± 22.57 recorded at 0,30,60,90, and 120 minutes after following glucose direction, respectively. It was interesting to note that Group III and Group IV demonstrated a greater tolerance to oral glucose than Group II. Group III had glycemic values of 134.75 ± 12.58, 176.75 ± 16.01, 168.50 ± 9.68, 153.50 ± 9.47, and 133.50 ± 16.42 at 0, 30, 60, 90, and 120 minutes after following glucose delivery. Out of the three Synergistic groups, 1stgroup (Group V) had a greater tolerance to oral glucose than Groups VI and VII.

Camel milk and *Hibiscus rosa sinensis* are both well-known for their antidiabetic properties (Mohamad *et al*., 2009; Ojiako *et al*., 2016). Both can lower the blood glucose levels of diabetic rats. By the end of the trial, the treatment groups' (III-VII) glycemic levels had returned to normal, comparable to the rats in the control group (I) (Fig. 1). This is in agreement with the study done by Venkatesh and Thilagavathi, (2008) using flower extract of *H. rosa sinensis* at doses 250 mg/kg and 500 mg/kg in the treatment of alloxan-induced diabetic rats. A study by AlKurd *et al.* (2022) suggested that daily intake of raw and pasteurized Camel milk helps reduce glycemic levels of type II diabetic patients. Sanadheera *et al*. (2021) concluded that tea made from the petals of *H. rosa sinensis* had anti-hyperglycemic and anti-hyperlipidemic properties, making it beneficial for managing diabetes. According to Mirmiran *et al*., (2017) diabetes patients' glycemic levels can be lowered by consuming 500 milliliters of camel milk daily. This may be because Camel milk contains insulin-like protein (Malik *et al*., 2012; Raj *et al*., 2023) and *H. rosa sinensis* extract can secrete insulin (Sachdewa *et al*., 2001; Vimala *et al*., 2008). Type II diabetes can be delayed by the presence of certain fatty acids found in Camel milk, which enhance the body's sensitivity to insulin (Shahriari *et al*., 2018). The amount of insulin in Camel milk is substantially larger than that of cow milk, making it more effective in decreasing blood glucose levels (Singh *et al*., 2006). The hypoglycemic effect of *H. rosa-sinensis* flowers is well-known in type II diabetes patients (Sharma *et al*., 2016).

The histological analysis of the pancreas and testis revealed that both Camel milk and *H. rosa sinensis* were able to reverse the damage caused by diabetes in treated rats (Fig. 2 and 3). Pillai and Mini (2016) found that when administered at a dosage of 25 mg/kg to diabetic rats, the ethyl acetate fraction of *H. rosa sinensis* petals retained the normal architecture of the islets of Langerhans, which includes an abundance of beta cells. A study conducted by Raj *et al*. (2023) demonstrated that feeding diabetic rats with

camel milk (50 ml/rat/day) reduces their glucose levels and reverses the pancreatic damage brought by diabetes. Additionally, Budin *et al*. (2018) found that giving *Hibiscus* extract to STZ-diabetic rats reverses testicular damage and improves testicular histology. In our previous research, Chauhan and Rani (2024) showed how *H. rosa sinensis*, administered at 125 mg/kg, lowers the glycemic levels of type I diabetic rats and enhances the structure of their pancreas.

Hibiscus reversed sperm damage in diabetic rats and significantly ($p<0.0001$) increased sperm count, viability, and motility (Table 1). This is consistent with the research conducted by Idris *et al*. (2012), which found that administering 100 mg/kg of *Hibiscus* extract to diabetic rats improved the quality of their sperm, resulting in significantly higher sperm concentrations ($p<0.05$), sperm motility ($p<0.001$), and a lower percentage of abnormal sperm ($p<0.05$) when compared to the diabetic control group. Camel milk also effectively improved sperm analysis and testicular damage and showed a protective effect on the reproductive system of rats (**Table 1**). This is in agreement with the study conducted by Gad *et al*. (2018), the administration of camel milk increased sperm motility%, live sperm%, sperm concentration, serum levels of progesterone and estrogen, as well as serum and testicular testosterone, and improved the rate of conception. However, it also significantly ($p\leq0.05$) decreased the percentage of sperm abnormalities. Mature rats fed camel milk had a considerable ($p\leq0.05$) increase in the width and lumen space of their seminiferous tubules on a histological level. Research on the synergistic benefits of Camel milk and *H. rosa sinensis* on the reproduction of diabetic rats was not accessible. However, the present study found that Camel milk and *H. rosa sinensis*, when taken in smaller amounts (Group V), worked synergistically to improve the reproduction of type II diabetic rats.

Conclusion

In treating type II diabetic Wistar albino rats, the effectiveness of Camel milk and *H. rosa sinensis* separately

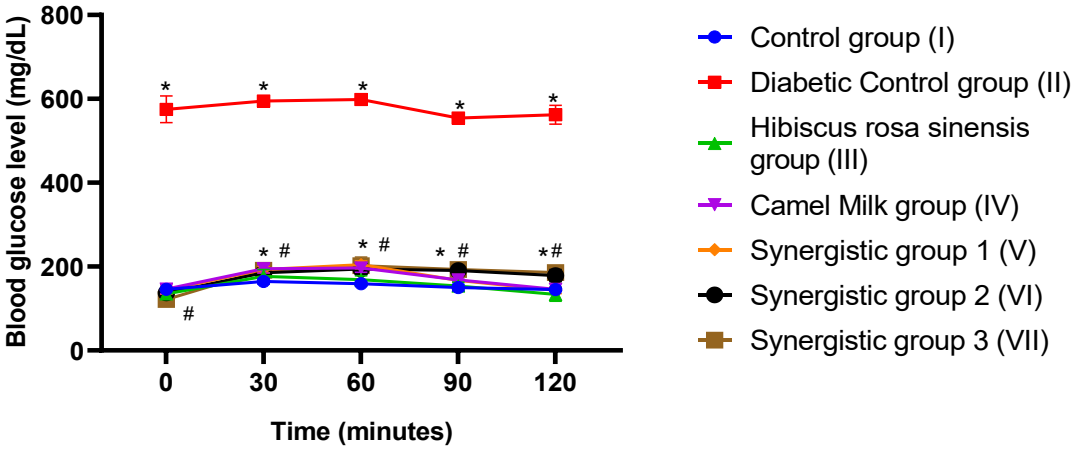

Fig. 4. *Oral glucose tolerance test of different groups after the 30 days of the experimental period. Data is represented as Mean ± SD, N = 4. *Values significantly differ from Group I at p < 0.05. #Values significantly differ from Group II at p < 0.0001*

Table 1. Sperm analysis of albino rats of different groups of type II diabetes

Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
Sperm count (million/ml)	26.50 ±1.29	0.75 ±0.95**	31.50 ±8.88##	14.25 ±5.56	10.75 ±4.11*	2.00 ±0.80**	1.62 ±0.47**
Motility (%)	77.48 ±1.73	18.57 ±3.54**	83.52 ±8.30##	59.47 ±4.91*##	61.39 ±12.33*##	34.06 ±2.70**#	33.62 ±3.82**#
Viability- (a) Live (%)	76.00 ±4.32	17.75 ±1.70**	84.21 ±8.98##	63.26 ±4.32##	63.70 ±17.12##	35.66 ±1.61**#	35.32 ±3.37**#
(b)Dead (%)	24.00 ±4.32	82.25 ±1.70**	15.78 ±8.99##	36.73 ±4.32##	36.27 ±17.08##	64.33 ±1.61**#	64.67 ±3.37**#

Note: *All values are represented as Mean ± SD, N = 4. *Values significantly differ from Group I at p < 0.05. **Values are highly significant (p < 0.0001) from Group I. #Values significantly differ from Group II at p < 0.05. ##Values are highly significant (p < 0.0001) from Group II.*

and synergistically showed that among the synergistic groups (V-VII), Synergistic group 1st (Group V) outperformed the other two synergistic groups (Group VI and VII), indicating that a lower dose of Camel milk and *H. rosa sinensis* was more effective than a higher dose. *H. rosa sinensis* and Camel milk may also be complementary therapies for type II diabetes. Further research is required to determine the specific mechanisms of action of *H. rosa sinensis* and Camel milk. Clinical trials are also required to assess these two's long-term benefits, safety, and effectiveness in people with diabetes.

Conflict of interest

The authors declare that they have no conflict of interest.

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