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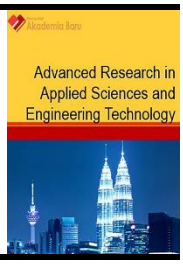

Testicular Damage and Abnormal Sperm Characteristic due to Chronic Hyperglycemia Exposure Restored by Polyphenol Rich Extract of *Hibiscus Sabdariffa Linn*

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ABSTRACT

Infertility is one of the complications that arise from chronic diabetes mellitus. As male infertility contributes to a large portion of the inability for couples to conceive, finding a remedy in preventing and treating diabetes-induced male infertility poses great importance. Roselle (*Hibiscus sabdariffa* Linn.) polyphenol-rich extract (HPE) has been previously reported to protect against hyperglycemia-induced oxidative stress in the early stage of diabetes. In this study, we investigated whether HPE is able to ameliorate the deterioration of sperm and testicular oxidative damage in chronic diabetic rats. Type 1 diabetes mellitus (insulin-deficient) was induced in rats with a single streptozotocin injection and left untreated for four weeks. Treatment with HPE (100 mg/kg body weight) and metformin (100 mg/kg body weight) commenced for the following four weeks while the non-diabetic control and diabetic control groups were both kept untreated for the whole eight weeks duration. Results revealed improvement of sperm quality on HPE-treated diabetic rats. Analysis of oxidative stress biomarkers in the testis was promising, with a reduction in malondialdehyde (MDA) and advanced oxidation protein products (AOPP). Also, the testicular level of antioxidant superoxide dismutase (SOD) and reduced glutathione (GSH) were elevated. The histological observation of the testis revealed active spermatogenesis along with restoration of spermatogenic cells in the lumens of the seminiferous tubules post-HPE treatment of diabetic rats. In conclusion, our study confirmed that HPE exhibits promising potential in treating diabetes-induced testicular oxidative damage and deterioration of sperm quality. Further study is needed to advance our understanding of the mechanisms underlying the action of HPE in modulating male reproductive dysfunction.

Keywords:
diabetes, oxidative stress, *Hibiscus sabdariffa*, testis, sperm

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1. Introduction

Diabetes mellitus (DM) is characterized by chronic hyperglycemia resulting from extensive damage of pancreatic beta cells that are responsible for producing insulin and/or heightened peripheral cells resistance towards insulin action [1]. Reproductive dysfunction is one of the significant secondary complications of DM [2]. It is estimated that 15% of couples worldwide are affected by infertility due to DM. Among those affected, approximately 40 - 50% of the cases are

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related to deterioration in sperm quality and other reproductive-related dysfunctions. According to Shi et al. [2], DM compromises male reproductive functions via disturbances to spermatogenesis, sperm maturation process, sperm fertilization capacity, penile erection as well as ejaculation.

Reactive oxygen species (ROS) are oxidizing agents produced by oxygen metabolism and are important for sperm functions. However, the failure to regulate the amount of ROS results in oxidative stress with adverse effects on reproductive wellbeing [3]. Oxidative stress, resulting from the imbalance between ROS and antioxidant defense systems, is a significant contributing factor towards male reproductive dysfunction. This is attributed to a substantial rate of cell division and intensified mitochondrial oxygen consumption in the testis. In a diabetic setting, oxidative stress is dramatically elevated via the generation of free radicals in multiple pathways, mostly triggered by the hyperglycemic and hyperlipidemic environment in the body [4]. The free radicals play a major role in the production of abnormal sperm, reduction in sperm count, and causing damage to sperm DNA. Sperm and testis are mainly constituted of polyunsaturated fatty acids (PUFA) than other cells, thus increasing their susceptibility to lipid peroxidation [5]. Furthermore, sperm and testis are equipped with poor antioxidant defense systems, making them more predisposed towards oxidative damage [5].

The rising concern of undesirable effects of synthetic drugs has rejuvenated the importance of traditional remedies in recent times. In traditional therapies, various medicinal plants have been used worldwide to manage DM-induced reproductive dysfunction. The practice is based on the perceived role of bioactive phytoconstituents that vary according to their nature and origin [6]. For example, raspberry extract treatment, reportedly rich with natural antioxidants, protected the male reproductive system against DM-induced dysfunction [7]. Similarly, male diabetic rats treated with *Artemisia judaica* extract led to enhanced sperm quality and reduced testicular oxidative damage via the upregulation of endogenous antioxidants production [8]. Treatment of *Momordica cymbalaria* and dolichandrone serrulata flower extracts both replicated similar protective effects on the male reproductive system in diabetic rat models [9, 10]. These findings are promising on the potential use of natural products in averting DM-induced reproductive dysfunctions.

A previous study suggested that *Hibiscus sabdariffa* Linn. or more commonly known as roselle, a perennial plant native in Malaysia, is generally rich in antioxidants [11]. Polyphenol-rich extract of roselle (HPE) makes an exceptional source of dietary phytochemicals as it is rich in anthocyanins, flavonoids, phenolic acids, vitamins, and various minerals [12]. The use of Roselle calyx as natural antioxidants, natural colorants, anticancer drugs, antibacterial, antifungal, anti-inflammatory, and anti-cholesterol seem to be promising and health-promoting [13]. While a previous study revealed the ability of HPE to protect against testicular damage during the early stage of diabetes in rat models [14], its ability to treat and prevent further development of male reproductive dysfunction in chronic diabetic condition remains elusive. Therefore, this study was designed to assess whether HPE treatment would ameliorate testicular damages and abnormal sperm characteristics due to prolonged diabetic conditions in the diabetic rats' model.

2. Methodology

2.1 Extraction of HPE

The dried calyces of *H. sabdariffa* Linn. UMKL-1 were obtained from HERBagus Sdn Bhd in Kepala Batas, Malaysia with a voucher specimen of PID050515-05 from Forest Institute of Malaysia. The polyphenol-rich extract of Roselle was prepared as previously described by Mohammed *et al* [15]. A total of 5 grams of ground calyxes were infused in 50 ml of HPLC-grade methanol and stirred in a 60 °C water bath for 30 minutes. The mixture was filtered with filter paper, and the filtrate was extracted

twice under the same procedure. The pooled extract was concentrated using a rotary evaporator (≤5°C, 20 mbar) and dissolved in 10 mL of deionized water. The resulting solution was partitioned successively with n-hexane (3 × 10mL) and ethyl acetate (3 × 10mL). The ethyl acetate soluble fraction was distilled to dryness using a rotary evaporator. The resulting polyphenol-rich extract was stored at -20°C in a dark container and freshly dissolved in water prior to animal treatment.

2.2 Animals

A total of 24 male *Sprague-Dawley* rats (200g-230g) aged eight weeks were obtained from the Laboratory Animal Resources Unit, Universiti Kebangsaan Malaysia. Animals were acclimatized to the experimental condition for one week before the experiment. Food and water were supplied *ad libitum* to the rats. All procedures with animals strictly adhered to the guidelines of the UKM Animal Ethics Committee (UKMAEC) (UKMAEC NO: FSK2015/2014/BALKIS/11-FEB./643-FEB.-2015-FEB-2018).

2.3 Experimental design and dosing schedule

A total of 18 rats were subjected to diabetes induction with a single intraperitoneal injection of streptozotocin (55mg/kg) at the beginning of the study [16]. After 72 hours, fasting blood glucose was measured using a glucometer. Rats with fasting blood glucose measured more than 15 mmol/L were considered diabetic. After being assigned into three groups; diabetic group (DM), diabetic treated with HPE (100 mg/kg) (DM+HPE), and diabetic treated with metformin (100 mg/kg) (DM+MET) all diabetic rats were left untreated for 28 days without any intervention before being assigned. Meanwhile, the non-diabetic rats were grouped as the control rats (NDM). NDM and DM groups were only received normal saline while treatment groups received HPE or metformin orally for 28 consecutive days (Day 29 until Day 56). Blood glucose level was measured on the 28^{th} and 56^{th} day and body weight, food as well as water intake were measured weekly.

2.4 Sample collection

At the end of the study, epididymal sperm was harvested for sperm analysis. The cauda epididymis was minced in 2 ml pre-warmed Hank's buffer salt solution (HBSS) at 37°C and centrifuged at 1,000 rpm, 4 °C for 3 minutes. The supernatant was immediately processed for sperm assessment. The reproductive organs were excised, washed with phosphate-buffered saline (PBS), and kept in 10% formalin for histology study. The remaining organs were stored at -40°C for oxidative stress determination.

2.5 Sperm Characteristics Analysis

Sperm characteristic analysis was performed based on guidelines lined by WHO [17] with modification. Sperm concentration and motility were determined using a Neubauer hemocytometer under a light microscope at ×100 magnification. Sperm concentration was expressed as 10^6/ml, while sperm motility was presented as the percentage of motile sperm. Abnormal sperm morphology was calculated using a thin smear of sperm suspension stained with Diff-Quik reagent and reported in percentage.

2.7 Oxidative stress biomarkers

One of the testes was subjected to tissue homogenization for the evaluation of oxidative stress biomarkers as described by Taib et al. [18]. Lipid peroxidation of testes homogenate was assessed using the method by Ledwoż et al. [19], where the thiobarbituric acid reacts with malondialdehyde (MDA), a by-product of lipid peroxidation to produce pink chromogen. The formation of thiobarbituric acid-reactive substances (TBARS) is directly proportional to MDA concentration in the sample that can be measured at 532 nm. Protein oxidation was determined by measuring advanced oxidation protein products (AOPP) according to the methods of Witko-Sarsat et al. [20]. AOPP is a dityrosine containing cross-linked protein products that act as a marker of protein oxidation due to oxidative stress. This method relies on the spectrophotometric measurement of the AOPP levels induced by chlorine oxidants (chloramines and hypochlorous acid) and calibration with chloramine-T solutions with the absorbance at 340 nm in the presence of potassium iodide. Antioxidant levels were determined by measuring superoxide dismutase (SOD) activity and reduced glutathione (GSH) levels. Measurement of SOD activities was determined according to Beyer & Fridovich [21] based on the principle that nitro-blue tetrazolium (NBT) undergoes photoreduction on exposure to light by superoxide radicals. The inhibition of NBT production determines the enzyme activity. GSH level was measured according to Ellman [22]. DTNB will react with GSH to produce yellow pyridine salts, TNB (5-thio-2-nitrobenzoic acid), and GS-TNB and is measurable spectrophotometrically.

2.9 Organ Histology

The testis was processed in a series of steps that includes dehydration, clearing, infiltration, and embedding. The paraffin block was sectioned into 4-5 µm thickness using a microtome and transferred to a glass slide. The dried slide was stained with Hematoxylin and Eosin (H&E) to observe the structural changes.

2.10 Statistical analysis

All results were expressed as a mean ± standard error of the mean (SEM). Statistical analysis was performed using GraphPad Prism 6.01 software (GraphPad, USA). Analysis of variance (ANOVA) was used for multiple comparisons and a p-value < 0.05 was considered for significant differences between the treatments.

3. Results & Discussion

3.1 Food and Water Intake

Average food and water intake measured at a one-week interval are illustrated in Fig. **1.** Pattern of food intake in all experimental groups throughout the study period
a: significantly different ($p < 0.05$) compared to the NDM group; b: significantly different ($p < 0.05$) compared to the DM group; c: significantly different ($p < 0.05$) compared to the DM+HPE group

and Figure 2, respectively. The diabetic rats demonstrated diabetic symptoms such as extreme reduction in weight aligned with polyphagia, polydipsia, and polyuria. Cell starvation due to the inability to obtain glucose as an energy source leads to increased food intake. This event further intensifies the hyperglycemic state. In addition, the osmotic load of hyperglycaemia leads to polyuria and polydipsia as glucose is excreted into the urine. Consequently, energy is obtained through the catabolism of protein and fat which eventually leads to a severe loss of body weight. This is caused

by sustained glycogenolysis and proteolysis to generate precursors for gluconeogenesis in hyperglycaemic status [23]. At the end of eight weeks, the DM+HPE group exhibited significantly ($p < 0.05$) lower food and water consumption compared to all DM groups. Observations of food and water intake reduction in rats treated with HPE are consistent with the antidiabetic properties of Roselle reported by Wisetmuen et al. [24].

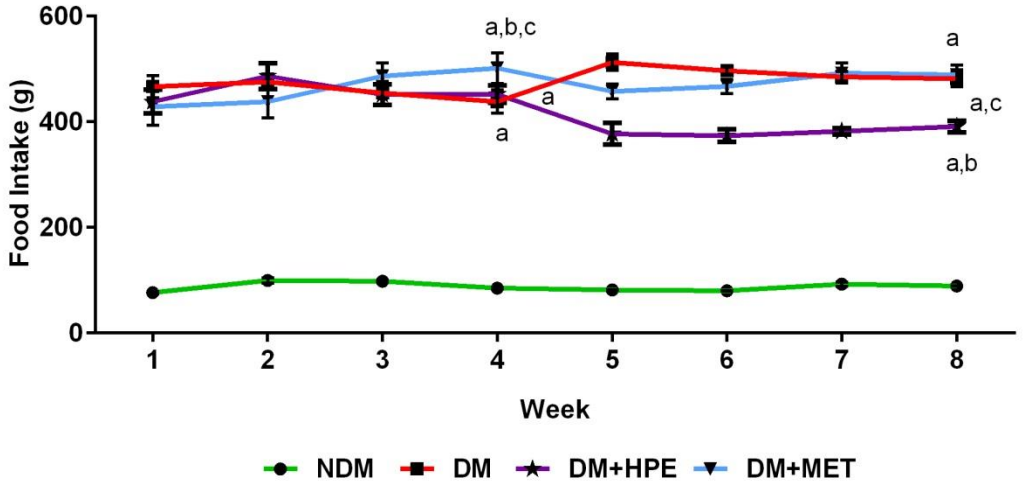

Fig. 1. Pattern of food intake in all experimental groups throughout the study period
a: significantly different ($p < 0.05$) compared to the NDM group; b: significantly different ($p < 0.05$) compared to the DM group; c: significantly different ($p < 0.05$) compared to the DM+HPE group

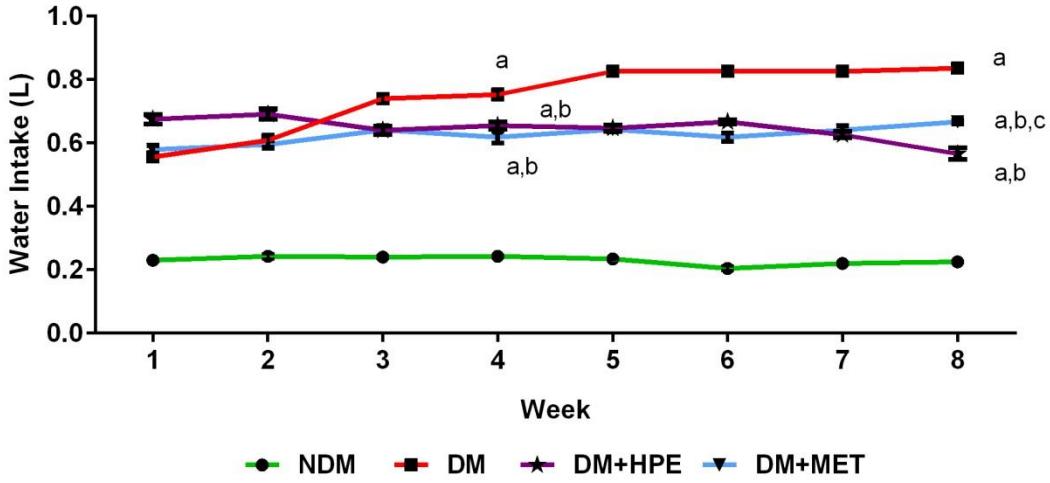

Fig. 2. Pattern of water intake in all experimental groups throughout the study period
a: significantly different ($p < 0.05$) compared to the NDM group; b: significantly different ($p < 0.05$) compared to the DM group; c: significantly different ($p < 0.05$) compared to the DM+HPE group

3.2 Blood Glucose Level, Testis and Body Weight

Toxic glucose analogs such as STZ accumulate in pancreatic β-cells and destroys the cells resulting in the inhibition of insulin secretion and subsequent increase in plasma glucose levels [25]. At the 8th week (

Fig. **3.** Fasting blood glucose level between groups throughout the study period.

Value depicted in mean ± SEM. a: significantly different ($p < 0.05$) compared to NDM group; b: significantly different ($p < 0.05$) compared to DM group

), all diabetic groups showed significantly higher fasting blood glucose compared with the non-diabetic group. In contrast, the DM+MET group demonstrated significantly lower fasting blood glucose ($p < 0.05$) compared to the DM group. The DM+HPE group also showed a lower fasting blood glucose than the DM group, but the difference was not significant ($p > 0.05$). A previous study [15] has shown that immediate commencement of HPE supplementation (duration of 8 weeks) after diabetes induction reduces glucose levels in diabetic rats. According to Wisetmuen *et al*. [24], this effect could be attributed to its ability to induce the generation of β- islets cells and increase insulin secretion. Throughout the study period, the DM+HPE group showed a consistent trend of lower fasting glucose level compared to the DM group. However, the non-statistically significant observation most probably due to chronic hyperglycemia caused β- islets cells progressed to an advanced degenerative state too severe to be reverted.

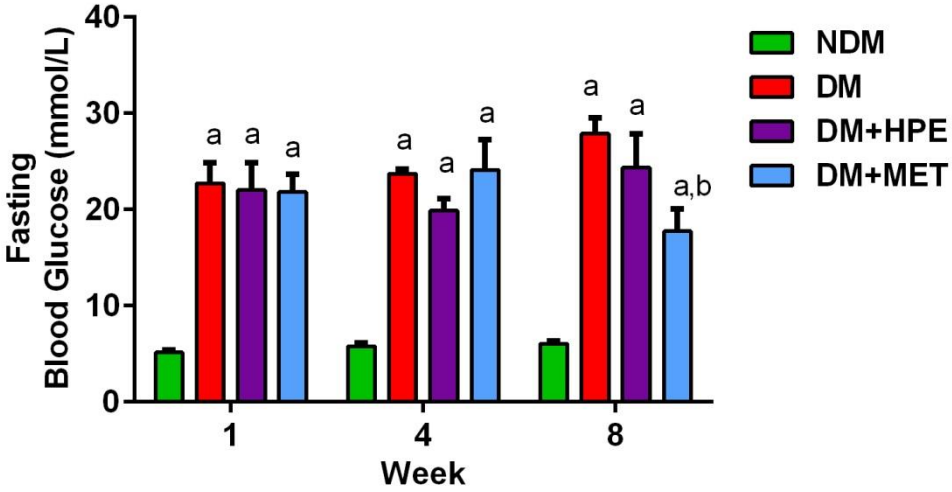

Fig. 3. Fasting blood glucose level between groups throughout the study period.
Value depicted in mean ± SEM. a: significantly different ($p < 0.05$) compared to NDM group; b: significantly different ($p < 0.05$) compared to DM group

The weight of testis and animals at the end of the study is depicted in Table 1. Both DM+HPE and DM+MET groups displayed higher testicular weight and body weight compared to the DM group. According to Kianifard et al. [26], reduction in testis weight is caused by testicular atrophy and/or shrinkage of the epididymis. In the diabetic state, gonadotrophins (LH and FSH) levels that influence the production of Leydig and Sertoli cells in the seminiferous tubules are reduced [26]. The downstream effect of low testosterone in STZ-induced diabetes [27] would cause arrested spermatogenesis and disrupt the proliferation of spermatogenic cells. In addition, the reduction in testicular weight is influenced by the density of the spermatogenic cells which affects the overall mass of the seminiferous tubule [28]. The administration of HPE improved the functional deficit and impeded the loss of testicular mass and body weight. This finding corroborates the reports from a previous study following the improvement of reduced testis weights and body weights after the administration of a flavonoid (i.e. quercetin) in diabetic rats [27].

Table 1
Testicular and body weights (g) in all groups at the end of the study period

Group	Weight (g)	
	Testes	Body

NDM	1.14± 0.05	305.33±14.21
DM	0.46± 0.12[a]	150.00±11.30[a]
DM+HPE	0.57±0.0.19	171.00±12.61[a]
DM+MET	0.61±0.22	171.33±18.35[a]

The values are expressed as mean ± SEM. [a]Significant difference as compared with the NDM control group at p <0.05.

Meanwhile, blood glucose level and body weight in HPE treated group did not exhibit significant differences compared to the DM group. In this study, the treatment was initiated four weeks after the onset of the diabetic condition. Most probably, Roselle treatment was not able to restore the pancreatic cell function after the destruction by STZ due to cell limitations. High regenerative capability of β-cell has only been reported during the neonatal period [29-30] in rats and mice. Hepatic β-cell has limited ability to regenerate new cells caused by apoptosis as a consequence of oxidative stress. The diabetic rats were left untreated for four weeks duration and this cause the pancreatic damage has already established and treatment of Roselle was unable to significantly reverse the oxidative damage and normalized the blood glucose levels.

3.3 Sperm Characteristics

Comparisons of sperm characteristics between experimental groups are shown in Table 2. Severe damage to the sperm characteristics was observed in all DM groups compared to NDM. However, there were no significant differences in sperm count, motility, and morphology between the DM, DM+HPE, and DM+MET groups. Omolaoye et al. [31] reported a complete absence of spermiogenesis in the testis of rats treated with STZ 60 mg/kg after eight weeks of induction.

Table 2
Sperm characteristics in all experimental groups

Group	Total Sperm Count (10^6/ml)	Sperm Motility (%)	Abnormal Morphology (%)
NDM	29.63±2.12	84.28±4.93	43.30±3.17
DM	6.58±2.93[a]	25.13±10.02[a]	87.93±4.49[a]
DM+HPE	5.92±3.50[a]	44.85±26.09[a]	79.48±8.15[a]
DM+MET	10.78±4.77[a]	53.05±20.05[a]	81.70±7.16[a]

The values are expressed as mean ± SEM. aSignificant difference as compared with the NDM control group at p <0.05.

The rats utilized in this study weighed approximately 200-230 g and eight weeks old at the start of the diabetes induction and were considered sexually matured [32]. Spermatogenesis lasts for 56-60 days in each cycle and occurs continuously in the seminiferous tubule. The sperm travels to the epididymis and persists in there for the continuity of the maturation process before entering the vas deference [33].

Spermatogenesis in DM rats is perturbed by the hyperglycemic state in diabetes before treatment. The sperm used for characteristic analysis were obtained from the epididymis. During the four weeks duration of treatment, only spermatogenic cells in the seminiferous tubule of testis responded to the treatment. In contrast, there was no response in the sperm in the epididymis. HPE treatment seems to exert a positive effect on the sperm in the seminiferous tubule as evident in the histological examination. However, the treatment did not exert the same positive effect on the sperm in the epididymis as indicated in sperm count, motility, and morphology results.

During the eight weeks observation, some of the sperm in the seminiferous tubule of testis migrated to the epididymis. It is suggested that sperm obtained in the epididymis and analyzed for characteristic features were produced in the testis before the treatment was initiated. Concurrently,

the sperm might have been damaged in the epididymis during exposure to diabetes. Sperm has low endogenous antioxidant capacity due to its compact cytoplasm and the high content of PUFA in the plasma membrane favors oxidative peroxidation. Unlike somatic cells, spermatozoa are unique because they are no longer capable of gene transcription. This situation is exacerbated by the lack of a cytoplasmic space to house antioxidant enzymes. The limited concentration of ROS-suppressing antioxidants, as well as high levels of unsaturated fatty acids in the sperm structure, renders it more susceptible to oxidative stress [4]. These were the possible reasons why four weeks of treatment of HPE does failed to reverse the damage of the matured sperm in the epididymis after extensive damage inflicted by the diabetic condition. The sperm morphology showed various forms of abnormalities and the sperm count and motility were severely affected. Sperm morphology observed in this study is depicted in **Error! Reference source not found.**. Head abnormality such as decapitation, headless, bent head, and pinhead while tail abnormality such as bent, coiled, curled, broken, and kinked tail was observed.

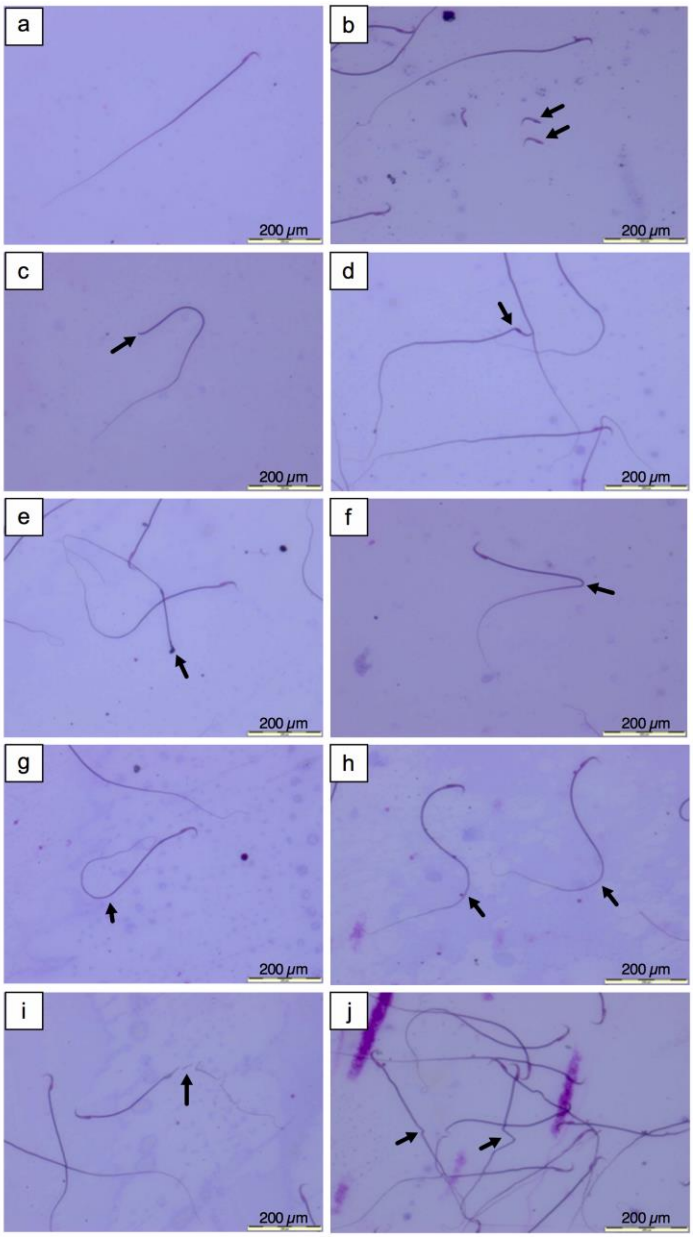

Fig. 4. Morphology of rats sperm using Diff-Quik staining in all experimental groups.

a) normal sperm; b) decapitated; c) headless; d) bent head; e) pinhead; f) bent tail; g) coiled tail; h) curled tail; i) broken tail; j) kinked tail

3.4 Level of Oxidative Stress Marker of Testis of in Experimental Groups

The effects of supplementation of HPE on MDA, AOPP, SOD activity, and GSH levels in testes in the experimental groups are shown in Table 3. There was a significant ($p<0.05$) increase in MDA level in the DM group compared to the NDM group. Notably, the concentration of MDA in DM+HPE groups was similar to NDM level. Besides, the concentration of MDA in treatment groups was significantly higher than the DM group and similar between treatment groups.

Table 3
The effects of supplementation of HPE on SOD, GSH, MDA and AOPP in testes

Group	Testis			
	SOD	GSH	MDA	AOPP
NDM	1.20±0.04	0.320±0.014	6.829± 1.169	70.00±4.163
DM	0.82± 0.07	0.332±0.035	58.83± 22.02[a]	100.3± 16.66
DM+HPE	1.12±0.15	0.413±0.034	9.271± 2.532 [b]	87.17±5.963
DM+MET	0.53±0.14 [a,c]	0.291±0.027 [c]	8.084±2.767 [b]	69.83±9.279

GSH, MDA, and AOPP are expressed as mmol/mg protein whereas SOD is expressed as U/min/mg protein. The values are expressed as mean ± SEM. [a] Significant difference as compared with the NDM control group at $p<0.05$. [b] Significant difference as compared with the DM control group at $p<0.05$. [c] Significant difference as compared with the DM+HPE control group at $p<0.05$

The MDA level in the DM group indicates the ongoing lipid peroxidation damage due to an increase in oxidative stress. Peroxidative damage is regarded as the most important cause of impaired testicular function leading to a variety of conditions including diabetes [34]. Thus, lipid peroxidation is one of the significant parameters to evaluate oxidative stress in testis. Based on our findings, supplementation of HPE to diabetic rats leads to a significant reduction of MDA. This finding is important given the abundance of fatty acid in the seminiferous tubule and its contribution to oxidative damage. The reduction of MDA in testis signals a positive outcome as testis is mainly made of PUFA and at risk of lipid peroxidation. Oxidative stress is detrimental to testis as it arrests spermatogenesis, lowers the testis mass, thus culminating in reduced weight of the organ.

AOPP level is a good indicator of oxidative stress status and degree of oxidative protein damage [35]. The AOPP levels for DM and DM+HPE groups were higher compared to the NDM group, whereas the levels were similar in DM+MET and NDM groups and no statistical difference was observed. Meanwhile, the HPE treatment was able to reduce the AOPP level compared to the DM group. The observation of lower MDA and AOPP levels is consistent with a study [36] that reported the antioxidative effects of Roselle extract in the red blood cells of diabetic rats.

SOD is one of the primary intracellular antioxidants which catalyzes the conversion of superoxide anion to hydrogen peroxide and oxygen. The SOD activity of the DM group was lower than NDM but the difference was not statistically significant. Meanwhile, SOD activity for the DM+HPE group was similar to the NDM group. However, the DM+MET group was significantly lower than both NDM and DM+HPE groups for SOD activity.

Interestingly, an increase of GSH levels was observed in DM and DM+HPE groups compared to the NDM group though the increment was not statistically significant. Mahjithiya and Balaraman [37] suggested that elevated antioxidant enzyme activity in the early stages of diabetes is a compensatory response due to increased oxidative stress. At the later stage of diabetes, reduction in

antioxidative activity may be due to the depletion of antioxidant activity negated by an excessive generation of reactive species in persistent hyperglycemia.

Furthermore, the elevated lipid peroxidation rate in this study may be attributed to the high PUFA found in testis. GSH is the most abundant low molecular weight antioxidant synthesized in cells [38]. GSH plays a critical role in the mitigation of oxidative damage. A deficit in this antioxidant leads to oxidant damage and greater lipid peroxidation which in turn causes cell damage. A concurrent decrease in GSH and increase of MDA was observed in this study, which is in agreement with the findings by Turgut *et al*. [39].

Endogenous antioxidants namely GSH and SOD measured in the study revealed a promising trend of improved production. A previous study by Budin et al. [28] posited that enhanced oxidative biomarkers levels can be associated with the high phenolic content found in the exogenous supplement. Antioxidative substances such as Roselle may contribute to the improvement of oxidative stress status by their capacity to scavenge free radicals and increase the levels of endogenous antioxidants. The trend of positive increment of antioxidant marker suggests that administration of HPE may have the potential to restore the antioxidant capability of GSH and SOD in diabetic conditions.

3.5 Histological Observation of Testis

Histological assessment was conducted to confirm the effectual restorative properties of HPE. Observation of the testicular tissue of DM groups showed atrophy of seminiferous tubule, loss of Leydig and Sertoli cells, depletion of centrally located spermatozoa, and disappearance of spermatids in the seminiferous tubule of diabetic rats. STZ-induced alkylation of DNA leads to necrosis of β cells [40]. Morphology of testis in DM+HPE displayed improvements on degenerative testicular damage occurring in the diabetic condition (Figure 5: E & F) and a similar event was observed in the NDM group. However, some degenerative tubules were detected but limited to a few tubules.

Testicular cell apoptosis and cell deaths are important events of diabetes and hyperglycemic conditions. It is well known that diabetes causes the reduction of spermatogenic cells and decreases the diameter of the tubules by cell apoptosis and seminiferous tubules atrophy. Histological study revealed a progressive reduction of density after diabetes induction. Hyperglycemia and diabetes mellitus may adversely cause cell death and lead to cell apoptosis in testicular tissues. These events may impair spermatogenesis and male infertility [41]. Likewise, this may also explain the reduction of organ weight as observed in the present study. This study demonstrated that the treatment with HPE for four weeks attenuated these changes and restored testicular structure. Therefore, the outcomes suggest restorative properties of HPE against oxidative stress-mediated testicular damage in diabetic rats.

4. Conclusion

We had previously proven that HPE was able to retain male reproductive wellbeing when treatment was commenced at an early stage of diabetes. To the best of our knowledge, the current study is the first to explore HPE effects on treating and limiting the development of reproductive dysfunction in chronic diabetic male rat models. This study elucidated the potential of HPE, extracted from roselle calyx, on treating diabetes-induced male reproductive dysfunction. The findings revealed that supplementation of HPE improved oxidative damage of sperm and restored testicular structure deterioration after chronic exposure to hyperglycaemic conditions. This outcome is believed to be contributed by HPE's high polyphenol and antioxidants content. However, the

mechanisms and pathways involved in the protective mechanism of HPE against diabetes-induced reproductive dysfunction were not elucidated. Further in-depth studies may consider investigating the possible mechanisms involved to further understand the modulation of HPE.

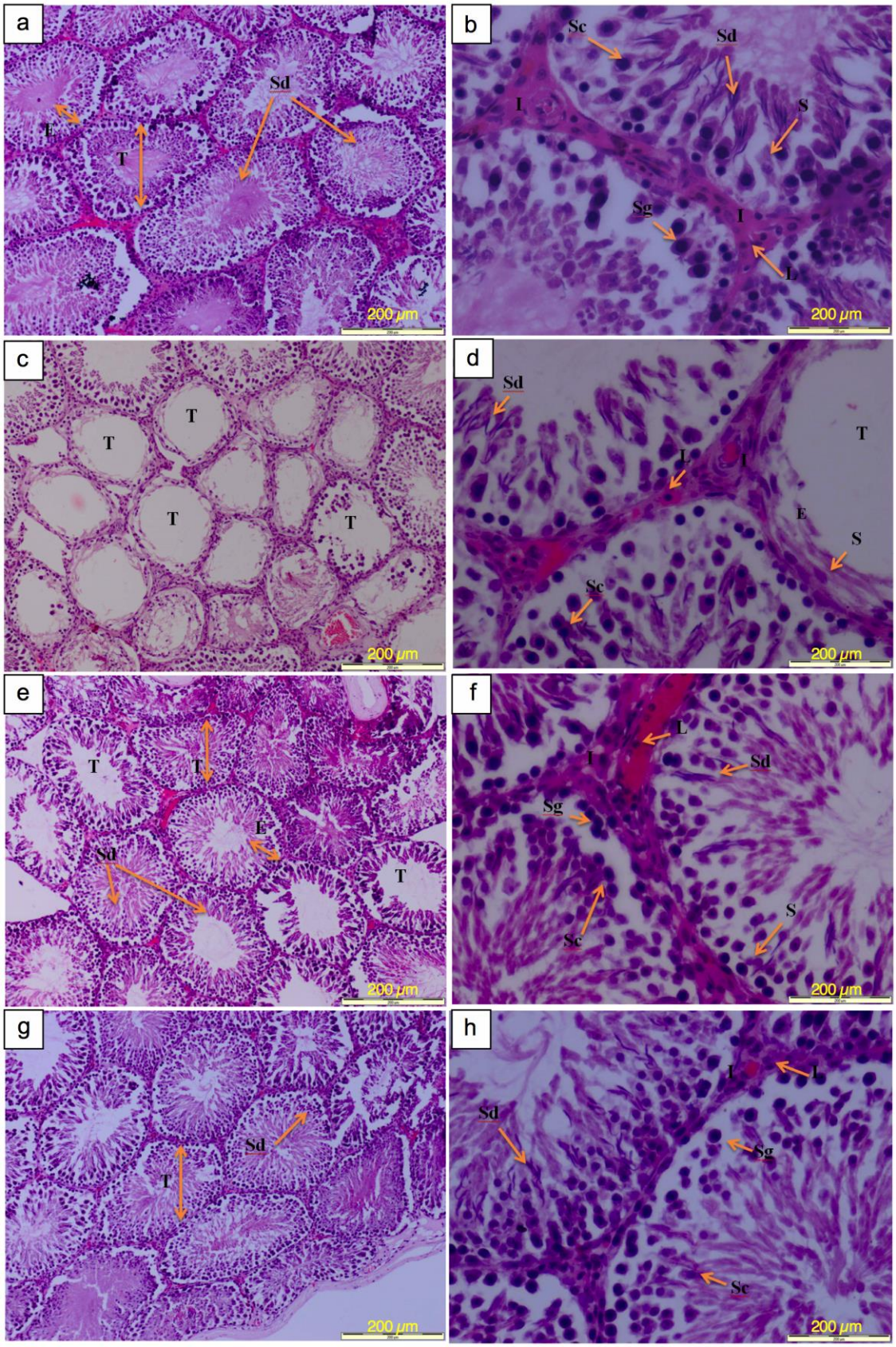

Fig. 5. Testis micrograph (H&E); NDM group (A&B), DM group (C&D) DM+HPE group (E&F), DM+MET group (G&H) in x100 and x400 magnification respectively. T=tubule L=Leydig cell S=Sertoli cells Sc=spermatocyte Sd=spermatid Sg=spermatogonia E=germinal epithelium I=interstium

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