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Polyphenol-Rich Extract of Roselle Improves Oxidative Stress Status, Renal Function and Structure of Diabetic Rat

(Ekstrak Rosel Kaya Polifenol Membaiki Status Tekanan Oksidatif, Fungsi dan Struktur Renal Tikus Diabetes)

FATIMA MOHAMMED YUSR, FATIN FARHANA JUBAIDI, SUMAYYAH BINTI ISMAIL & SITI BALKIS BUDIN*

Center of Diagnostic, Therapeutic and Investigative Studies, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia

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ABSTRACT

Diabetic nephropathy is a progressive chronic renal disease, that leads to renal failure mediated by oxidative stress. Roselle or *Hibiscus sabdariffa* is well-known for its health benefits and is rich in polyphenols with high antioxidant activities. Limited studies evaluate the effects of polyphenol-rich extract of roselle (HPE) on the development of diabetic nephropathy. Hence, this study aimed to determine the effect of HPE in the renal of diabetic rats focusing on oxidative stress, renal function, and structural changes. Type 1 diabetes mellitus was induced in adult male Sprague-Dawley rats which were then divided into three groups: untreated diabetes (DM), HPE-supplemented diabetic rats (DM+HPE), and metformin-treated diabetic rats (DM+MET). Non-diabetic rats (NDM) served as normal controls. All rats were left untreated for four weeks followed by another four weeks of treatment, respectively. At the end of the study, all rats were sacrificed whereby blood was collected for creatinine level analysis, and kidneys were collected for oxidative stress and antioxidant markers as well as histological analyses. Data showed that HPE reduced blood glucose and creatinine levels significantly and improved catalase (CAT) activity. HPE supplementation also reduced the lipid and protein oxidation process, marked by significant reductions in malondialdehyde (MDA) and advanced oxidation protein products (AOPP). Moreover, HPE supplementation improved renal structure, especially in reducing glomerulus degeneration and renal tubule damage. In conclusion, this study suggests that HPE is able to reduce renal damage by mitigating oxidative stress and improving antioxidant status that potentially prevents the progression of diabetic nephropathy in diabetic rat model.

Keywords: Diabetes; diabetic nephropathy; oxidative stress; polyphenol; roselle

ABSTRAK

Nefropati diabetes adalah penyakit kronik buah pinggang yang progresif yang membawa kepada kegagalan buah pinggang yang diperantarai oleh tekanan oksidatif. Rosel atau *Hibiscus sabdariffa* terkenal dengan khasiat dan manfaat kesihatannya serta kaya dengan polifenol yang mempunyai aktiviti antioksidan yang tinggi. Tidak banyak kajian yang dilakukan untuk mengkaji kesan ekstrak rosel kaya polifenol (HPE) terhadap perkembangan nefropati diabetes. Oleh itu, kajian ini bertujuan untuk mengenal pasti kesan HPE terhadap buah pinggang tikus diabetes yang memberi fokus kepada tekanan oksidatif serta perubahan fungsi dan struktur renal. Diabetes melitus jenis 1 diaruh pada tikus Sprague-Dawley jantan dewasa dan kemudian dibahagikan kepada tiga kumpulan; diabetes yang tidak dirawat (DM), DM dengan suplementasi HPE (DM+HPE) dan DM dengan metformin (DM+MET). Kumpulan tikus bukan diabetes (NDM) berfungsi sebagai kawalan normal. Semua tikus dibiarkan tanpa rawatan selama empat minggu dan diikuti dengan empat minggu rawatan mengikut rawatan masing-masing. Pada akhir tempoh kajian, tikus dikorbankan dan darah diambil untuk penentuan aras kreatinin, manakala ginjal diambil untuk penentuan tekanan oksidatif, antioksidan serta penilaian histologi struktur renal. Data menunjukkan HPE menurunkan aras glukosa darah dan kreatinin dengan ketara dan meningkatkan aktiviti katalase (CAT). Suplementasi HPE juga menurunkan proses oksidasi lipid dan protein yang ditandai dengan penurunan aras malondialdehid (MDA) dan produk protein pengoksidaan lanjutan (AOPP) yang ketara. Malah suplementasi HPE juga turut membaik pulih struktur renal terutamanya mengurangkan degenerasi glomerulus serta kerosakan tubul renal. Kesimpulannya, kajian ini mencadangkan bahawa HPE berupaya mengurangkan kerosakan buah pinggang dengan mengurangkan tekanan oksidatif dan meningkatkan status antioksidan yang berpotensi untuk menghalang perkembangan nefropati diabetes dalam model tikus diabetes.

Kata kunci: Diabetes; nefropati diabetes; polifenol; roselle; tekanan oksidatif

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by elevated blood glucose levels, known as hyperglycemia (Zhang et al. 2020). DM affects multiple organ systems and can lead to serious complications such as cardiovascular disease, neuropathy, nephropathy, and retinopathy. The presence of DM magnified the burden of illness, reduced living quality, and increased the mortality rate (Mohd Nor et al. 2022). The global incidence of DM prevalence is on the rise whereby according to the International Diabetes Federation report (2021), around 643 million adults (20-79 years) are expected to be diabetic patients by 2030 and 783 million by 2040.

Diabetic nephropathy (DN) is a progressive kidney disease and a complication of DM. It is a leading cause of chronic kidney disease (CKD) and end-stage renal disease worldwide (Samsu 2021). DN occurs when high blood glucose levels and hypertension damage the blood vessels in the kidney, impairing their ability to filter waste and excess fluid from the body. Over time, this can lead to kidney failure if not properly managed (Qi et al. 2017). The progression of DN is typically divided into five stages based on the level of kidney function and proteinuria. The stages are (1) glomerular hyperfiltration, (2) incipient nephropathy or early renal damage, (3) microalbuminuria, (4) overt proteinuria or macroalbuminuria, and (5) end-stage renal disease (ESRD) (Hernandez et al. 2022). Chronic hyperglycemia enhances the polyol pathway, activating protein kinase C (PKC) and excess generation of advanced glycation end products (AGEs) that results in increased reactive oxygen species (ROS) production which lowers antioxidant capacity ending with oxidative stress condition (Singh, Winocour & Farrington 2010). ROS is a main culprit in DN progression whereby it induces oxidative damage on proteins, lipids, and DNA, resulting in glomerular injury, interstitial fibrosis, microvascular dysfunction, and inflammation which aggravate renal damage (Vodošek Hojs et al. 2020).

Intense and rigorous therapy to normalize blood glucose levels and avoid hyperglycemia complications is always associated with unfavorable side effects (Corathers, Peavie & Salehi 2013), therefore there is an increased interest in the use of natural products as a source of diabetes treatment (Abdullah et al. 2018). Polyphenols reportedly exhibited hepatoprotective effects and could diminish oxidative stress and improve antioxidant activities in the eye lenses of diabetic rats (Justino et al. 2017; Sedlak et al. 2018). Polyphenols extracted from roselle or *Hibiscus sabdariffa* have been reported to be rich in antioxidative compounds such as epigallocatechin-gallate, flavonoids (Jamrozik, Borymska & Kaczmarczyk-Żebrowska 2022), anthocyanins, phenolic acids, and organic acids which contribute to its medicinal properties (Mohammed Yusof et al. 2018a; Riaz & Chopra 2018). Furthermore, these bioactive components, either alone or in combination, showed significant antioxidant, anti-inflammatory, and anti-carcinogenic characteristics, and may also help to improve diabetes condition and its complications (Ojulari, Lee & Nam 2019).

A study reported that polyphenol-rich extract of roselle (HPE) exhibited antihyperglycemic and antihyperlipidemic properties and improved diabetic cardiovascular complications via decreased oxidative stress, cardiomyocyte hypertrophy, and myocardial fibrosis in the cardiac of diabetic rats (Mohammed Yusof et al. 2018a). In addition, HPE has shown potential against diabetic vasculopathy and showed improvement in serum lipid profiles and regulation of glucose metabolism (Peng et al. 2011) as well as eliminated oxidative damage in the testis of diabetic rats (Budin et al. 2018). Diabetic nephropathy is among the main complications of diabetes and its pathogenesis involves oxidative stress which leads to alteration in renal function and structure. Previous research documented that 4 weeks of the untreated condition led to the development of diabetic nephropathy in the type 1 diabetic rat model (Kaikini et al. 2020). There are limited studies aimed to evaluate the effects of HPE on the development and progression of diabetic nephropathy. Due to the potential of HPE in eliminating various diabetic disorders, hence, this study aims to determine the effect of HPE on oxidative stress status, renal function, and structural changes in the renal diabetic rat model.

MATERIALS AND METHODS

PREPARATION OF POLYPHENOL-RICH EXTRACT OF ROSELLE (HPE)

Dried calyces of roselle were procured from HerBagus Sdn. Bhd, Kepala Batas, Penang, Malaysia, and were stored at 4 ºC. A specimen was sent to the Forestry Research Institute of Malaysia (FRIM) for identification and the voucher number of the specimen is PID050515-05. The roselle calyces were ground, and the resulting powder was stored in a refrigerator at -20 ºC for further use.

The extraction process followed the method outlined by Peng et al. (2011). Roselle calyces powder was soaked in methanol and constantly stirred in a water bath for 30 min at 60 °C. The extract was then filtered using Whatman no. 4 filter paper, and the residue underwent the same procedure two more times. The extracts were pooled and evaporated using a rotatory evaporator at ≤5 °C on 20 mbar pressure. The dried extract was solubilized in 10 mL of deionized water (pH 2.3). The solution was then partitioned three times with 10 mL of each of n-hexane and ethyl acetate, with the ethyl acetate soluble fraction subsequently evaporated to dryness using a rotary evaporator (Buchi, Switzerland). The final crude extract was stored at -20 ºC. For animal feeding, it was dissolved in distilled water freshly before supplementation.

ANIMALS

Adult male Sprague-Dawley rats (230-250 g) were used in this study and underwent one week of acclimatization period before the experiment began. They were housed at the animal research laboratory in hygienic conditions with temperatures between 25 and 28 ºC with 12 h light/dark cycles. The rats were fed a standard pellet diet and provided with free access to drinking water throughout the study. This study was approved by the Animal Ethics Committee, Universiti Kebangsaan Malaysia (UKMAEC) under the Ethics Number: FSK/2020/Siti Balkis/25-Nov./1120-Dec.-2020-2022.

DIABETIC INDUCTION AND TREATMENT

Before the induction of diabetes, all rats were fasted overnight. Diabetes was induced using freshly prepared streptozotocin (STZ) (Sigma Chemicals, ST. Louis, Missouri, USA) at a dose of 55 mg/kg body weight via a single intraperitoneal injection (Mohammed Yusof et al. 2018b). After 72 h, blood glucose levels were measured to confirm the diabetic status, and rats with a glucose level above 15 mmol/L were selected for this study (Mohammed Yusof et al. 2018a). Diabetic rats were randomly divided into three groups (n=8): diabetic (DM), diabetic treated with HPE (DM+HPE) (100 mg/kg), and diabetic treated with metformin (DM+MET) (150 mg/kg). The dosages for HPE and metformin were according to Mohammed Yusof et al. (2020) and Majithiya and Balaraman (2006), respectively. Meanwhile, non-diabetic rats (NDM) served as a control group (n=8). All rats were left untreated for a four-week duration to allow the development of diabetic nephropathy (Kaikini et al. 2020), and then HPE and metformin treatments were commenced via oral force-feeding, daily for another four consecutive weeks (Jubaidi et al. 2021). At the end of the treatment period, rats were fasted overnight, blood was collected via cardiac puncture, and rats were sacrificed to excise the kidneys. The procedure was performed under ketamine/xylazine cocktail anesthesia (KTX) (Jubaidi et al. 2021).

ASSESSMENT OF PLASMA GLUCOSE AND SERUM CREATININE

Blood glucose was measured using the D-Glucose test Kit (Megazyme, Ireland). The test kit utilizes the oxidation of glucose-by-glucose oxidase into D-gluconic acid and hydrogen peroxide, which is then reacted with p-hydrobenzoic acid and 4-aminoantipyrine (in the presence of peroxidase) to produce a quinoneimine dye. This dye can be detected using a spectrophotometer at 510 nm. Serum creatinine was measured according to Jaffe's reaction method (Jaffe 1886), wherein an orange colour compound is produced from the reaction of creatinine and picric acid in an alkaline medium (Toora & Rajagopal 2002). After an incubation period at room temperature for 15-20 min, the intensity of the coloured compound is proportional to the creatinine level in the sample. The colour intensity was measured at 520 nm by a spectrophotometer.

KIDNEY HOMOGENIZATION

The kidney tissue was weighed and carefully chopped using a sterile scalpel, then immersed in 0.1 M of PBS (phosphate buffer solution) solution at a ratio of 1:10 (w/v). Subsequently, the kidney tissue was homogenized with a homogenizer for 5 min. The resulting homogenate was centrifuged at 10,000 rpm for 15 min at 4 ºC, and the supernatant was collected and stored at -80 °C for further use.

OXIDATIVE STRESS EVALUATION

Superoxide dismuatase (SOD) activity was determined according to Beyer and Fridovich (1987) by measuring the reduction of NBT-diformazan. Superoxide ions (O^{2-}) transform NBT into NBT-diformazan, and tissue SOD reduces O^{2-} which lowers the NBT-diformazan formation. NBT-diformazan exhibits a dark purple colour and was measured at a wavelength of 560 nm. The method used for measuring catalase (CAT) activity is described by Aebi (1984), in which the degradation rate of hydrogen peroxide into water and oxygen by CAT activity was determined by measuring the absorbance decrease at 240 nm per minute. Meanwhile, reduced gluthathione (GSH) levels were determined by measuring the interaction between 5,5'dithiobis-2-nitrobenzoic acid (DTNB) and GSH molecules, producing a yellow compound known as 5-thionitrobenzoic acid (TNB). The chromogen produced is directly proportional to the GSH concentration in the sample and can be measured at a wavelength of 415 nm (Ellman 1959).

The colorimetric method described by Stocks and Dormandy (1971) was performed to measure malondialdehyde (MDA) levels. In this assay, thiobarbituric acid (TBA) reacts with the MDA molecule at 100 ºC for 30 min, resulting in a pinkish chromogen that can be detected at a wavelength of 532 nm. The level of advanced oxidation protein product (AOPP) was measured according to Witko-Sarsat et al. (1996) method. In this procedure, AOPP will oxidize I^- to I^{-3} under acidic conditions, producing a yellow chromogen that can be detected at a wavelength of 340 nm.

HISTOLOGICAL EVALUATION

The excised kidneys were fixed in formalin and processed using Leica Tissue Processor (Leica Biosystem, Germany), where the tissue underwent dehydration through processing in various concentrations of alcohol. Subsequently, the processed tissues were embedded in paraffin to form paraffin tissue blocks and sectioned at a thickness of 3-5 µm before being stained with Hematoxylin and Eosin for

microscopic histological evaluation. The images were processed using the ImageJ image analysis software (Fiji software version 1.53c). By using the freehand tool in the ImageJ software, the outer margin was outlined, and the area was automatically calculated by the software. The resulting area represents the area of interest, measured in square units (Cheng et al. 2012).

STATISTICAL ANALYSIS

Statistical analysis was conducted using one-way ANOVA followed by Tukey multiple comparison tests. All data analysis was performed using the GraphPad Prism software version 9.0.0 and expressed mean ± SEM. The significance level was set at $p< 0.05$ with a sample size of n=8 for each group.

RESULTS

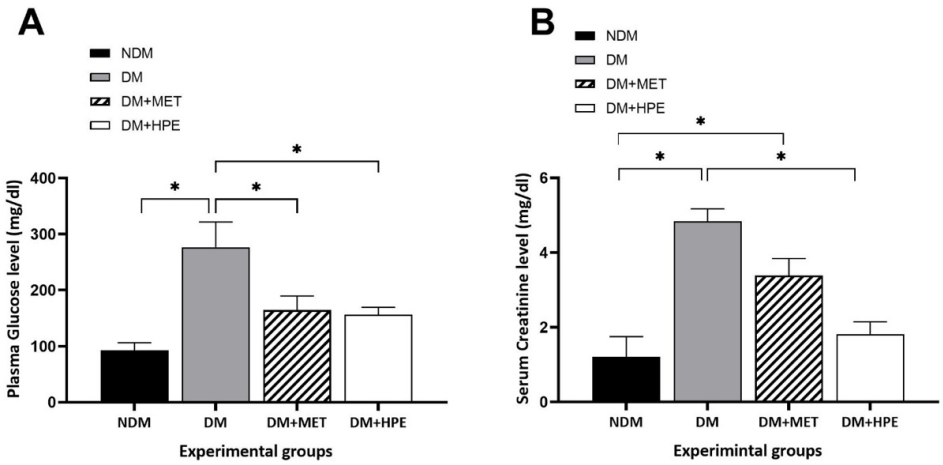
PLASMA GLUCOSE AND SERUM CREATININE LEVELS

A comparison of the plasma glucose levels between all experimental groups is exhibited in Figure 1(A). In this study, the DM group showed significantly higher glucose levels ($p<0.05$) when compared with the NDM group. Interestingly DM+HPE group showed a significantly lower plasma glucose level when compared with the DM group ($p<0.05$). However, there is no significant difference seen between the DM+HPE group compared to both NDM and DM+MET groups. The serum creatinine level among the experimental groups is shown in Figure 1(B). Serum creatinine levels in the DM group showed significantly higher ($p<0.05$) when compared with the NDM group. DM+HPE group exhibited a significant reduction in serum creatinine level compared with DM ($p<0.05$) but showed no significant difference when compared to NDM and DM+MET groups.

RENAL ANTIOXIDANT AND OXIDATIVE STRESS STATUS

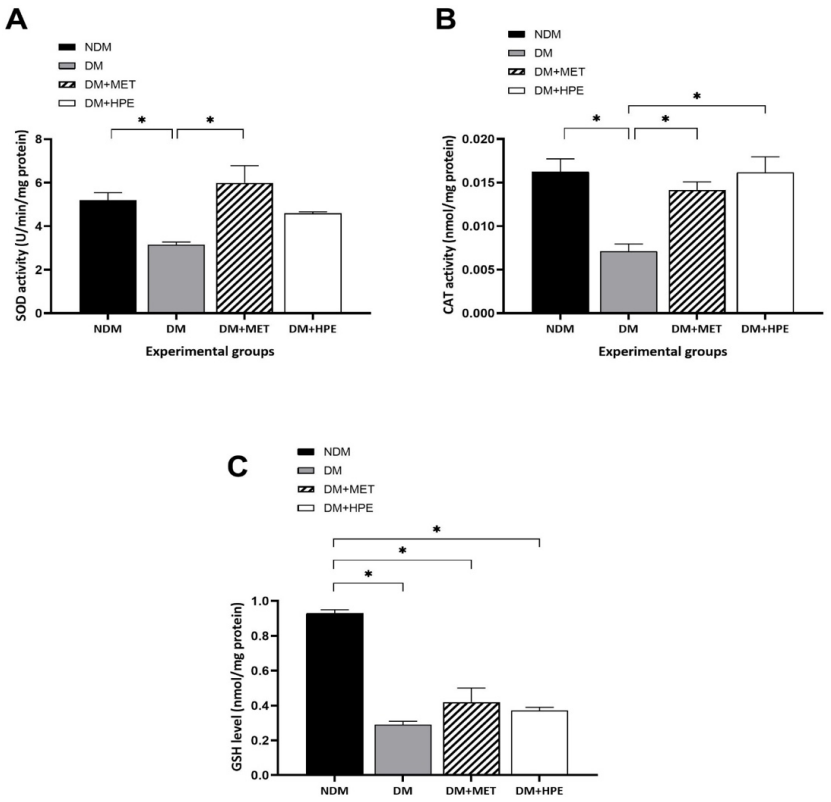
The antioxidant enzyme activities are illustrated in Figure 2. The SOD activity in the DM group showed significantly lower ($p<0.05$) when compared with the NDM group. While there was an increase in renal SOD activity in the DM+HPE group, there was no significant difference compared to the DM group. In addition, there were no significant differences in the SOD activity between the DM+HPE group compared to the NDM and DM+MET groups as displayed in Figure 2(A). The CAT activity in the DM group also showed a significantly lower ($p<0.05$) when compared with the NDM group. However, a significantly higher CAT activity was exhibited in the DM+HPE group compared with DM ($p<0.05$), but no significant difference was observed in the CAT activity when compared with NDM and DM+MET which is demonstrated in Figure 2(B). Meanwhile, diabetes induced significantly lower GSH levels compared to the NDM group ($p<0.05$). However, the DM+HPE group displayed an insignificant different GSH level compared with the DM and DM+MET groups as presented in Figure 2(C).

On the other hand, the oxidative stress status is presented in Figure 3. The MDA levels in the DM group showed significantly higher ($p<0.05$) when compared with the NDM group. HPE supplementation was able



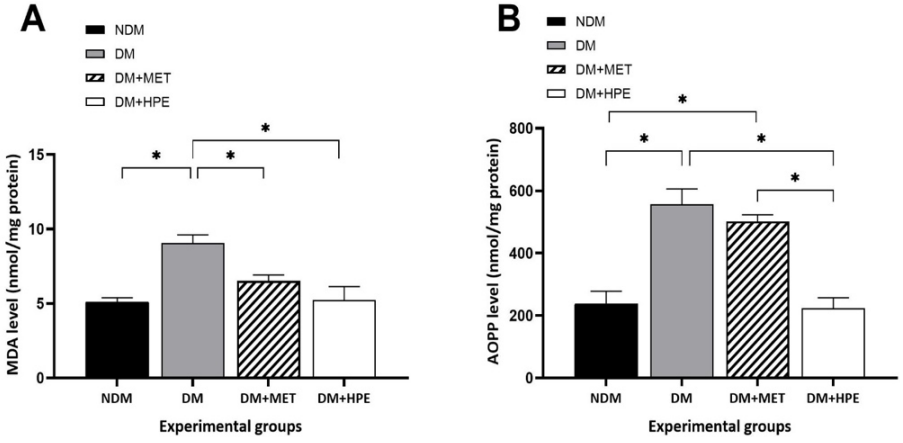
n=8 in all groups and data was expressed as mean ± SEM. *: $p<0.05$

FIGURE 1. Comparison of the (A) plasma glucose levels and (B) serum creatinine levels between the control (NDM), diabetic (DM), metformin-treated diabetic (DM+MET), and HPE-treated diabetic (DM+HPE) groups. Results showed improvement in both plasma glucose and serum creatinine levels in diabetic rats after HPE supplementation



n=8 in all groups and data was expressed as mean ± SEM. *: p<0.05

FIGURE 2. Comparison of the antioxidant status between the control (NDM), diabetic (DM), metformin-treated diabetic (DM+MET), and HPE-treated diabetic (DM+HPE) groups. (A) Superoxide dismutase (SOD) activity, (B) catalase (CAT) activity, (C) levels of reduced glutathione (GSH). HPE supplementation improved antioxidant status in the kidney of diabetic rats



n=8 in all groups and data was expressed as mean ± SEM. *: p<0.05

FIGURE 3. Comparison of (A) malondialdehyde (MDA) and (B) advanced oxidation protein product (AOPP) between the control (NDM), diabetic (DM), metformin-treated diabetic (DM+MET) and HPE-treated diabetic (DM+HPE) groups. Both renal MDA and AOPP levels decreased with HPE supplementation to diabetic rats

to significantly reduce the MDA levels in the DM+HPE group compared to DM ($p<0.05$). However, there is no significant difference in the MDA levels of the DM+HPE group compared to NDM and DM+MET as shown in Figure 3(A). Meanwhile, the AOPP levels in the DM group showed significantly higher ($p<0.05$) when compared with the NDM group. However, the DM+HPE group has significantly lower AOPP levels compared with DM and DM+MET groups ($p<0.05$), and an insignificant difference in AOPP levels compared with NDM as illustrated in Figure 3(B).

HISTOLOGICAL OBSERVATION

The kidney histological structures in all experimental groups are depicted in Figure 4. The kidney section of NDM rats (4(A)) exhibited a normal glomerulus structure with an intact form of both distal convoluted tubules (DCT) and proximal convoluted tubules (PCT). Conversely, the kidney section of the DM group (4(B)) showed degeneration of the glomerulus along with tubular necrosis of DCT and PCT. Interestingly, the kidney sections of both DM+MET and DM+HPE groups (4(C) and 4(D)), respectively, showed less degradation of the glomerulus and renal tubules.

The average glomerular area size for each experimental group is presented in Figure 5. Diabetes caused a significant reduction ($p<0.05$) in the glomerular size as observed in the DM group compared to the NDM group. HPE supplementation was able to normalize the glomerular size as seen in DM+HPE group whereby they were observed significantly larger compared with DM group ($p<0.05$).

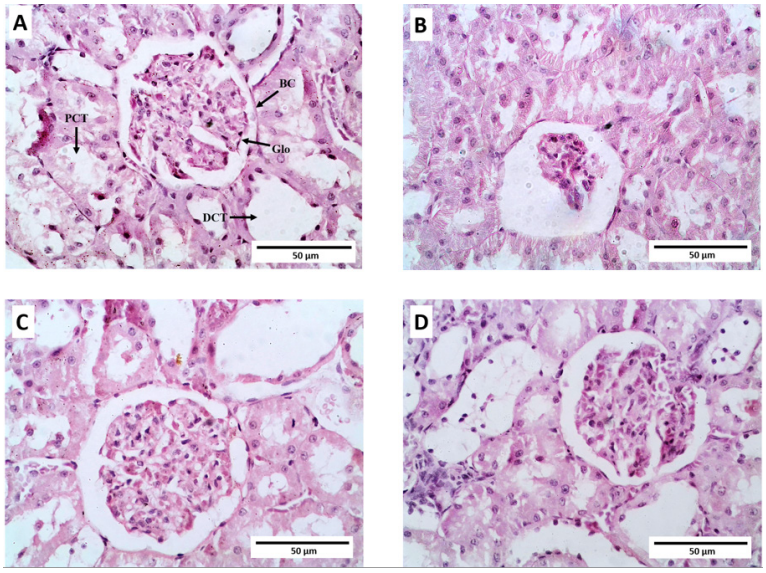
DISCUSSION

Roselle is a plant widely cultivated in tropical and subtropical countries. It is known as a rich source of polyphenols which contain polyphenolic acids, flavonoids, stilbene, and ligans (Sapian et al. 2022), alongside anthocyanins, and organic acids (Izquierdo-Vega et al. 2020). Many studies have stated that polyphenols have medicinal properties such as antihyperglycemic effects (Yuan et al. 2019). Moreover, roselle polyphenols have been reported as an effective agent against diabetic cardiac dysfunction (Mohammed Yusof et al. 2018a), diabetic kidney fibrosis (Huang et al. 2016), and diabetic testicular damage (Budin et al. 2018). This implies that roselle has an attenuating effect on hyperglycemia and diabetic complications, making it a natural option for developing a new diabetic renal protective agent.

In this study, the diabetic rats were left untreated for four weeks followed by another four weeks of HPE treatment. Results showed that all diabetic rats have significantly higher plasma glucose levels. STZ induction destroys the β-cell of the pancreas leading to absolute insulin deficiency that results in hyperglycemia. Interestingly, HPE supplementation was shown to reduce plasma glucose levels in diabetic rats, likely due to the content of organic acid, anthocyanin, flavonoids, and polyphenols (Bule et al. 2020). Mohammed Yusof et al. (2018b) stated that several previous research studies demonstrated that the roselle aqueous extract blocked the enzymes that hydrolyze carbohydrates (α-amylase and α-glucosidase), which delayed carbohydrate digestion, decreased glucose absorption, and controlled hyperglycemia. It was also proposed that HPE works as an antioxidant to scavenge the produced ROS and prevent more of β cell damage (Pannangpetch et al. 2013).

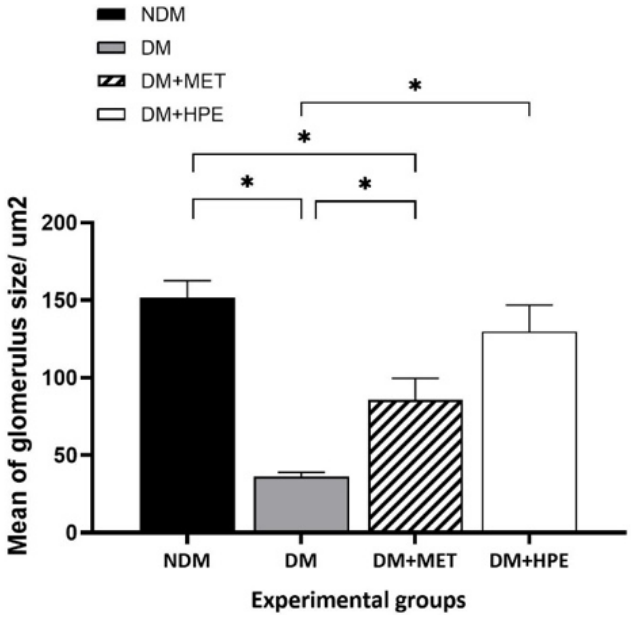
The type 1 diabetes mellitus model is among the popular models used to study diabetic nephropathy condition. The glycemic status and duration of diabetes determine the development rate and progression of diabetic nephropathy towards the advanced stage. Many studies started the treatment at the beginning of the diabetic confirmation to prevent the initiation and development of diabetic nephropathy. There are limited studies to investigate the ability of HPE to treat or ameliorate the progression of diabetic nephropathy in diabetic nephropathy rats. According to Kaikini et al. (2020) after four weeks duration, the untreated type 1 diabetic rats showed the features of early-stage diabetic nephropathy. In this study, the HPE supplementation was given after the rats were left untreated for four weeks. The findings showed a dramatic elevation of serum creatinine levels in diabetic rats due to sustained hyperglycemia (Salgado et al. 2010; Zhao & Fan 2020), and HPE supplementation successfully normalized the serum creatinine levels. This result is in line with many previous studies that stated the ability of HPE to decrease the effect of inflammation and oxidative stress, which can protect the kidney from oxidative damage and inflammation (Guerreiro et al. 2022). Ellagic acid, ferulic acid, and caffeic acid are among polyphenol components proved by prior studies to have the ability to act as anti-inflammatory agents and antioxidants (Zhou et al. 2019; Wang et al. 2017). Furthermore, HPE was also found to maintain renal function and modify the renal vascular response, both of which help raise the glomerular filtration rate and improve creatinine clearance (Rodríguez-Fierros et al. 2021).

In this study, DM successfully induced oxidative damage as shown by the marked decline of CAT, SOD activities, as well as lower GSH levels, accompanied by a significant increase in both MDA and AOPP levels. Hyperglycemia can stimulate the overproduction of ROS, which suppresses CAT activity due to an imbalance in ROS levels (Mohammed Yusof et al. 2018 a). Hyperglycemia also decreases SOD activity due to renal cell damage caused by oxidative stress (Rodríguez-Fierros et al. 2021). The decreased GSH level is attributed to the severe oxidative stress caused by hyperglycemia (Mi et al. 2022), which oxidizes the reduced GSH and lowers the cellular antioxidant capacity (Budin, Ismail & Pek Lian 2013).



PCT: proximal convoluted tubular, DCT: distal convoluted tubular, BC: bowman's capsule. Glo: glomerulus

FIGURE 4. Light microscopy observation (Hematoxyline & Eosin stain; ×400 magnification) of renal tissues from indicated rat groups. Section (A) was taken from the NDM rat group showing normal architecture of renal tissue with intact bowman's capsule, glomerulus texture, and normal renal tubules, (B) was taken from a DM rat's kidney showing severe degeneration of the glomerulus, and renal tubular necrosis, and (C) and (D) were taken from the kidneys of DM+MET and DM+HPE, respectively, showing less degree of glomerulus and renal tubular degeneration



n=8 in all groups and data was expressed as mean ± SEM. *: p<0.05

FIGURE 5. Comparison of the glomerular area size among experimental groups, control (NDM), diabetic (DM), metformin-treated diabetic (DM+MET) and HPE-treated diabetic (DM+HPE). HPE supplementation protected the glomerulus from diabetes-induced degeneration

In contrast, this study found that CAT, SOD activities, and GSH levels increased following HPE supplementation. Flavonoids such as epigallocatechin gallate, anthocyanin, and ferulic acid present in roselle can reduce the oxidative stress status (Ahn, Kim & Ha 2010; Mohammed Yusof et al. 2018b). In addition, citric acid and ascorbic acid may enhance the antioxidant enzyme activity (Rodríguez-Fierros et al. 2021). Moreover, due to the ability of HPE to scavenge oxygen free radicals and decrease lipid peroxidation, it results in less renal cell damage (Wang et al. 2020). Besides, the potency of polyphenols acts as a metal chelator to reduce ROS and upregulate SOD activity (Lakey-Beitia et al. 2020). Another study conducted by Lee et al. (2009) reported that polyphenol treatment increased GSH levels in polyphenol-treated diabetic rats.

HPE supplementation resulted in reduced MDA and AOPP levels, suggesting due to potent antioxidants in polyphenols that can neutralize ROS, and reduce oxidation of lipid and protein (Budin et al. 2018). Anthocyanins, flavonoids, and gallic acid are the main antioxidative stress components in HPE, which suggests its contribution to the reduction in MDA and AOPP levels (Malik et al. 2017; Tang et al. 2019).

Diabetes could cause significant damage to renal tissue and reduce the glomerulus area following hyperglycemia-induced oxidative damage to its cellular and structural components. Due to its antihyperglycemic and antioxidative enzyme properties, it is suggested that HPE could ameliorate the structural changes resulting from diabetes (Zhou et al. 2019). Respectively, phenolic acids, flavonoids, stilbene, and lignans in HPE could alleviate the histological changes that occur in different stages of diabetic nephropathy by reducing glomerular hypertrophy, glomerular sclerosis, preventing the disruption of podocytes and basement membrane thickening, in addition to limiting the mesangial cells migration (Sapian et al. 2022).

In this study, the ability of HPE supplementation to reduce renal damage in diabetic rats was compared with metformin, whereby it was showed that HPE exerted a comparable renal protective effect compared to with metformin. Metformin treatment reduces plasma glucose levels by increasing insulin sensitivity in peripheral tissue, blocking hepatic gluconeogenesis, and decreasing intestinal absorption of glucose (Kawanami, Takashi & Tanabe 2020). The reduction in serum creatinine emphasizes the findings of Corremans et al. (2018), who stated that metformin can ameliorate renal function. Meanwhile, Budin, Ismail and Pek Lian (2013) reported that the action of metformin in reducing hepatic glucose synthesis, promotion of skeletal muscle glucose absorption, and increasing glucose uptake by adipose could raise the SOD activity, while the high activity of CAT was explained by Corremans et al. (2018,) who found that metformin reduces renal cell damage due to its hypoglycemic effect, counteracts the hepatic glucagon, and inhibits gluconeogenesis in hepatocytes. Diniz Vilela et al. (2016) stated that metformin can aggregate NADPH, which is important for maintaining a normal GSH level. Therefore, the anti-hyperglycemic and antioxidative effects of HPE are suggested to be the main contributor to its ability to protect against diabetes-induced renal structural changes.

CONCLUSION

This study elucidated that the supplementation of HPE lowered serum creatinine levels and improved oxidative stress status while ameliorating the structural changes in the kidneys of diabetic rats. One limitation of this study is the reliance on serum creatinine only to assess renal function, which did not reflect the full renal function result for the study. Despite this limitation, the present study provides valuable insights into the therapeutic potential of HPE to limit the progression of diabetic nephropathy. However, the effects of HPE on diabetic nephropathy development have not been fully discovered. Therefore, the effects of HPE on oxidative stress and inflammation pathways for diabetic nephropathy development should be further explored to provide more information on the effects of HPE in ameliorating diabetic nephropathy.

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*Corresponding author; email: balkis@ukm.edu.my