Hypoglycaemic and Protective Effects of *Benincasa hispida* Aqueous Extract in Streptozotocin-Induced Diabetic Rats

(Kesan Hipoglisemik dan Pelindungan Ekstrak *Benincasa hispida* pada Tikus Teraruh Streptozotocin)

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ABSTRACT

Benincasa hispida (BH) contains a range of compounds which play important roles in treating human illnesses. This study was undertaken to evaluate the hypoglycaemic and protective effects of BH fruit on diabetes mellitus (DM) rats. The dose of 250 mg/kg of *B. hispida* aqueous extract (BHE) is the most effective dose in decreasing blood glucose level (BGL). After eight weeks of treatment, the BGL of BHE-treated DM rats (21.4±1.0 mmol/l) was found to be significantly lower than control DM rats (30.1±3.8 mmol/l). The weight of BHE-treated rats was also higher compared to the other DM groups. Overall, the biochemical evaluation of BHE-treated rats such as lipid profile, liver function test, kidney function test and HbA1c showed an improvement in biomarker values compared to the other groups. There were enhancements in liver and kidney structures of the BHE-treated group compared to those with metformin treatment, which indicated the protective effects of BH on the impaired organ structure. These findings suggest that BHE treatment exerts hypoglycaemic and protective effects in DM by decreasing the BGL, improving weight and biochemical parameters, as well as protecting liver and kidney from serious damage.

Keywords: *Benincasa hispida*; diabetes mellitus; hypoglycaemic; protective effect

ABSTRAK

Benincasa hispida (BH) mengandungi pelbagai jenis sebatian yang berperanan penting dalam merawat penyakit manusia. Kajian ini dijalankan untuk menilai kesan hipoglisemik dan perlindungan buah BH terhadap tikus yang berpenyakit diabetes mellitus (DM). Sebanyak 250 mg/kg ekstrak akues *B. hispida* (BHE) adalah dos paling berkesan yang menurunkan paras gula darah (BGL). Selepas lapan minggu rawatan, paras BGL tikus yang dirawat dengan BHE (21.4±1.0 mmol/l) turun secara signifikan berbanding tikus DM kawalan (30.1±3.8 mmol/l). Berat tikus yang dirawat dengan BHE juga tinggi berbanding lain-lain tikus daripada kumpulan DM. Secara keseluruhannya, penilaian biokimia tikus yang dirawat dengan BHE seperti profil lipid, ujian fungsi hati, ujian fungsi ginjal dan HbA1c menunjukkan penambahbaikan nilai biopenanda berbanding lain-lain kumpulan. Struktur hati dan ginjal tikus yang dirawat dengan BHE juga lebih baik berbanding yang dirawat dengan metformin, menunjukkan kesan perlindungan BH terhadap struktur organ yang terjejas. Keputusan kajian mencadangkan rawatan BHE mempunyai kesan hipoglisemik dan perlindungan daripada DM dengan menurunkan BGL, memperbaiki berat badan dan parameter biokimia serta melindungi hati dan ginjal daripada kerosakan yang serius.

Kata kunci: *Benincasa hispida*; diabetes mellitus; hipoglisemik; kesan perlindungan

INTRODUCTION

Diabetes mellitus (DM) is a type of metabolic disorder disease suffered by vast majority of population around the world which is considered as one of leading causes of death. Globally, the prevalence of DM in adults was estimated to rise from 463 million people in year 2019 to 578 million by 2030 and 700 million by 2045 (Saeedi et al. 2019). The National Health and Morbidity Survey (NHMS) stated that in 2019, the prevalence of DM in Malaysia was escalated (18.3%) compared to year 2015 (17.5%). DM is defined by hyperglycaemia, and it is associated with a complete or relative deficiency in the insulin action, or insulin secretion or both (ADA 2015). DM patients are susceptible to have other health problems if the disease is not properly treated or controlled. The primary cause of hyperglycaemia is impairment of insulin secretion, insulin action, or both, also chronic hyperglycemia is accompanied by long-term impairment, dysfunction, and failure of several organs (WHO 2015). Fortunately, a sustained decrease in hyperglycaemia would reduce the risks of emerging microvascular diseases and their secondary complications (Davies et al. 2018).

Appropriate non-pharmacological management of DM, such as healthy lifestyle, proper physical activities and proper nutrition, will provide huge benefits to diabetic patients. Typically, oral synthetic antidiabetic drugs are being used as a treatment for type 2 DM. Unfortunately, they can lead to serious side effects including hypoglycaemic coma and hepatorenal disturbances (Manandhar Shrestha et al. 2017); furthermore, their intake is not recommended during pregnancy (Atta-ur-Rahman & Zaman 1989). However, the use antidiabetic drug such as metformin may contribute to low levels of serum vitamin B_{12} and red blood cell folate (Luciano-Mateo et al. 2017) and less maternal weight gain (Priya & Kalra 2018). Considering these issues, ongoing research has been carried out by researchers around the world to identify safer and more effective hypoglycaemic agents. Consumption of medicinal plants is believed to help diabetic patients to lower their blood glucose and reduce DM secondary complications.

Winter melon, or *Benincasa hispida* (BH), are grown primarily for its fruits, and it is usually recognized for its nutritional and therapeutic properties, especially in Asian countries (Zaini et al. 2011). Traditionally, BH fruit has been used as laxative, diuretic, tonic, aphrodisiac, cardiotonic, urinary calculi, blood disease, epilepsy, schizophrenia and other psychological disorders, jaundice, dyspepsia, fever, and menstrual disorders (Al-Snafi 2013). Despite the consumption as nutritious vegetables, a ripe BH fruit also used as functional food ingredient and chosen by ancient health practitioners as a medicinal ingredient (Zaini et al 2011). According to the Index of Nutritional Quality (INQ) data, BH has been valued as a high-quality vegetable (Mingyu et al. 1995). It has natural bioactive and therapeutic agents with multiple bioactivities, which give huge benefits to human. Recently, polyphenols are very popular bioactive compounds among other phytochemicals because of their potent antihyperglycaemic effects, safe and causes no side-effects.

Some studies had been carried out to evaluate the effects of BH fruits on DM (Mahatma et al. 2015, 2014; Mohana Rupa & Mohan 2013). A study investigated hypoglycaemic effect of aqueous extract of BH stem by Mahatma Rupa and Mohan (2013) reported that 200 mg/kg of the extract was significantly reduced the blood glucose level of alloxan monohydrate induced-diabetic rabbits. While another study of anti-diabetic potential of methanolic extract of BH in dexamethasone-induced insulin resistant rats showed a promising effect of BH in controlling BGL. In addition, BH extract also assist in counteracting the instability of lipid profile. Mahatma et al. (2015) showed the antidiabetic potential of methanolic extract of BH in streptozotocin (STZ) induced diabetic rats. They found that the BH extract significantly decreased BGL of the rats, serum triglycerides and very low-density lipoprotein (VLDL) levels. Hence, the present study is conducted to investigate hypoglycaemic and protective effects of BH aqueous extract in streptozotocin-induced diabetic rats and to explore the effects of BH in restoring the impairment of liver and kidney of DM rats. Moreover, the biochemical analysis of the bloods can assist the investigation diabetes complications by comparing them with observations of the structure of kidney and liver.

MATERIALS AND METHODS

SAMPLE PREPARATION

Mature BH was purchased from the local market in Kota Bharu town, Kelantan, Malaysia. At the initial stage of preparation, the fruit (specimen number 11709) was cleaned, and its pulp was homogenized with a food processor and mixed with distilled water in 1:1 ratio. Then, the mixture was slowly heated (60±2 °C) for 30 min and was continuously stirred. Then, the mixture was cooled at room temperature before being filtered using

a muslin cloth and centrifuged (5000 rpm, 10 min). The resulting clear juice was placed in a specimen bottle and was frozen (-20 °C, 12 h) before being freeze-dried (IlShin, Korea) for further analysis.

EXPERIMENTAL ANIMAL

Male albino Sprague-Dawley (SD) rats with eight weeks of age were obtained from Animal Research and Service Centre (ARASC), USM, Kubang Kerian Campus, Kelantan, Malaysia. The animals were kept in ARASC at an ambient temperature of 24-30 ºC with a 12 h dark and light cycle, respectively. The animals were fed with commercial pelleted diet and water *ad libitum*. The present study was approved by the animal ethics committee of USM (USM/Animal Ethics Approval/(91) (539)).

INDUCTION OF DIABETES

Freshly prepared 45 mg/kg body weight of streptozotocin (STZ) in 0.1M citrate buffer (pH 4.5) was injected intraperitonially to a group of overnight fasted rats to induce diabetes. After a week, the severely diabetic rats with fasting blood glucose (FBG) greater than 13.8 mmol/L were selected as suggested by Kesari et al. (2006).

HYPOGLYCAEMIC ACTIVITY OF BHE IN DM RATS FOR EIGHT WEEKS

Twenty-four rats were equally divided to four group, which were as follows; Group A: normal control (distilled water); Group B: BHE-treated DM rats (BHE 250 mg/kg); Group C: metformin-treated DM rats (metformin 175 mg/kg) and Group D: control DM rats (distilled water). All groups were given oral treatment of a single dose for eight weeks. The BGL and weight for each rat were measured every two weeks. In measuring the BGL, blood was withdrawn via tail vein prick and estimated using a glucometer. After completing the treatment, the rats were fasted for 8 h and anaesthetized with sodium pentobarbital, 50 mg/kg intra-peritoneal. Blood was withdrawn by cardiac puncture for biochemical analysis of lipid profile, HbA1c, liver function test (LFT), and renal function test (RFT).

HISTOPATHOLOGICAL ANALYSIS

The kidney and liver of each rat were removed immediately after sacrifice and then were fixed in 10% formalin solution and stored in room temperature. The tissues were then processed using automated tissues processor machine and then were embedded in molten paraffin wax to form blocks. The tissue specimens' blocks were sectioned at 4 μm using a rotary microtome and stained with hematoxylin and eosin (H&E). Histopathological observation was made using light microscope (Olympus, Japan).

LETHAL DOSAGE

Six animals per group, three females and three males (180 - 220 g) were administered a single dose of 2500 mg/kg (10 times of effective dose) of BHE orally. Another group of rats were given a single dose of 3750 mg/kg (15 times of effective dose) of BHE. The rats were observed for gross behavior and toxic effects at 2, 6, 12 and 24 h. Food consumption, feces and urine were also observed at the same interval of time (Kesari et al. 2006).

STATISTICAL ANALYSIS

The data were represented as means ± SEM. All statistical analyses were carried out by GraphPad Prism 5 software. One-way analysis of variance (ANOVA) followed by 'Tukey's multiple comparison test' was used to assess the differences. A p value <0.05 were considered as statistically significant.

RESULTS AND DISCUSSION

HYPOGLYCAEMIC ACTIVITY OF BHE IN SEVERELY DIABETIC RATS FOR EIGHT WEEKS

Preliminary study had been done to evaluate the hypoglycaemic effects of *Benincasa hispida* aqueous extract (BHE) in normal and DM rats with a single dose range of 100, 250, and 500 mg/kg given by oral gavage (Fatariah et al. 2016). The study was conducted on fasting blood glucose (FBG) and oral glucose tolerance test (OGTT) referring to the method by Singh et al. (2007). OGTT study on normal and DM rats has been done to measure the blood glucose level of normal and DM rats in response to different treatments in 3H. The outcomes showed normal rats treated with 250 mg/kg of BHE demonstrated similar trend close to normal control rats. The dose of 250 mg/kg BHE also decreased the BGL of severely DM rats after 3 h. For this reason, 250 mg/kg dosage of BHE was selected for the eight weeks' treatment of DM rats. The eight weeks' duration of study was decided base on the results obtained from four weeks of treatment. At Week 4 (W4) (Figure 1) DM rats treated with BHE showed same blood glucose level with control DM (without treatment). This situation was led to a decision to extend the treatment up to eight weeks.

Moreover, extensive period of diabetes induction will significantly alter the kidney and liver structure and it will display clear observation of impaired structure under the microscope after staining.

From the results, at Week 2 (W2), the BGL of BHE-treated rats was significantly lower than the control DM rats by 35% (p<0.01) (Figure 1). At Week 8 (W8), the BGL of rats with BHE treatment significantly decreased by 28.8% (p<0.05) with respect to control DM rats. Metformin-treated rats also showed significant reduction in the BGL by 50.4% compared to the control DM group at W8 (p<0.001). The weight of BHE-treated rats significantly decreased by 17% at W2 compared to the initial week, i.e. W0 (Figure 2). Even though the weight of BHE-treated rats gradually decreased week by week, there were improvements when comparing it with the control DM and metformin groups. At the end of the study (i.e. W8), the weight of BHE-treated rats was higher than those treated with metformin and untreated DM rats. This finding indicated that metformin does not prevent weight loss in DM rats, which agrees with another study which assessing glycaemic control of metformin mixed with honey in DM rats (Erejuwa et al. 2011). One of the main signs of DM is weight loss which could be caused by loss of appetite and tissue protein, as well as increased muscle waste (Adisa et al. 2011). In addition, dehydration and catabolism of fats and proteins in DM rats can also lead to the loss of body weight (Hakim et al. 1997).

The improvement of BGL in diabetic rats treated with BHE in the OGTT study indicating augmented glucose tolerance due to increased glucose transport and utilization. The changes in the BGL showed the hypoglycaemic effects of BH in the treated diabetic group which may be caused by the presence of various bioactive compounds including gallic acid in the extract (Fatariah et al. 2014). The ability of gallic acid in scavenging free radicals and inhibiting lipid peroxidation has resulted in decreasing the BGL and increasing the plasma insulin levels (Punithavathi et al. 2011a). Increased insulin levels could be due to the stimulatory effect of gallic acid, thus potentiating the existing β-cells in diabetic rats (Punithavathi et al. 2011b). As BH belongs to Curcurbiteae family, the antidiabetic effects may also be associated with its natural polysaccharides (Simpson & Morris 2014).

For lipid profiles, both metformin and BHE-treated DM rats showed lower value of total cholesterol than the control DM group (Table 1). The levels of serum triglycerides in rats treated with metformin and BHE were significantly lower than the normal rats (p<0.05). On the other hand, the Low-Density Lipoprotein (LDL) level of rats treated with metformin and BHE were slightly lower than the control DM rats. Hyperlipidaemia associated with DM may be a consequence of an accelerated biosynthesis of hepatic triglyceride and the secretion of very low-density lipoprotein (VLDL) (Adeneye et al. 2010). The changes in the results of lipid profiles (Table 1) were supported by the histological improvement of fat content in the livers of DM rats (Figure 3). Other cucurbit fruits are also effective as antidiabetic treatment, at the same time contribute to significantly improved lipid profiles of animal diabetic models (Simpson & Morris 2014).

In liver function test (LFT), serum activities of alanine transaminase (ALT) liver enzyme in rats treated with BHE comparable to the control normal rats. In contrast, ALT in the untreated DM rats and metformin groups were significantly elevated compared to the normal rats (p<0.05). Alkaline phosphatase (ALP) liver enzyme in the BHE-treated group and control DM group were significantly higher than the normal group. Total protein and serum activities of ALT and ALP liver enzymes are useful biomarkers of liver abnormality. The decreased total protein in STZ-induced DM rats may be due to microproteinurea which is an important clinical indicator of diabetic nephropathy; it may also be due to the increase of protein catabolism (Almdal & Vilstrup 1988). The elevated ALT and ALP levels in STZ-induced DM rats' serum are associated with liver damage. This is due to the leakage of these biomarker enzymes from the liver cytosol into the blood stream (Navarro et al. 1993). The increased serum activities of these enzymes were reduced by BH administration. BHE-treated rats also showed significant reduction in the ALP levels (Table 1). The elevated enzymes in the DM rats might lead to diabetic complications, such as gluconeogenesis and ketogenesis (Gosh & Suryawansi 2001). The present study has shown that the BHE treatment improved the restoration of these enzymes to the normal level.

In renal function test (RFT), there were no significant changes in the creatinine and K levels in all treatment groups compared to the normal rats. Both serum urea and creatinine levels are the convincing biomarkers that indicate the kidney function. Escalation of serum urea and creatinine levels are commonly occurring in patients with diabetes (Mulec et al. 1990). The BHE treatment reduced the serum urea level in DM rats by comparison with the untreated DM group, albeit not significant (Table 1). An abnormality in the serum creatinine level also indicates the impairment of renal function (Perrone et al. 1992).

In the present study, serum creatinine levels in all groups did not changed significantly.

Higher amounts of serum K in DM might be caused by kidney insufficiencies, which are associated with hyperkalemia. Hyperkalemia is a potentially serious metabolic problem caused by the failure of the kidneys to excrete K and incapacitate the movement of K from the circulation into the cells (Hollander-Rodriguez & Calvert 2006). The serum K level in BHE-treated DM rats was reduced although not significant (Table 1). The improvement in the K level in BHE-treated rats indicates that the bioactive compounds in BH are capable to protect kidneys from serious problems. This is because phenolic acid might have acted as a modulator of K distribution by improving the K level in plasma (de Sotillo & Hadley 2002).

On the evaluation of glycosylated haemoglobin (HbA1c), all groups showed a significant increase of HbA1c compared to the normal control rats. However, the BHE-treated rats lower in HbA1c levels compared to the metformin-treated rats. HbA1c is also considered as a biomarker for a risk factor for DM vascular complications such as retinopathy, nephropathy, and other complications (Lyons & Basu 2012). The American Diabetes Association (ADA 2015) recently outlined the use of HbA1c as a diagnostic criterion of prediabetes (5.7%) and DM (>6.5%). In this study, the HbA1c value of STZ-induced DM rats was significantly higher than the normal group (Table 1). However, the HbA1c value of BHE-treated rats was slightly lower than those of metformin-treated and untreated DM rats. The improvement of HbA1c might be due to the improved glycaemic control produced by BH. A study demonstrated a significant reduction in the HbA1c level in DM rats treated with *Solanum torvum* extract which contains 4.78% (w/w) gallic acid (Gandhi et al. 2011). Thus, the reduction of HbA1c in BHE-treated rats may be due to the presence of gallic acid in the extract (Fatariah et al. 2014).

HISTOPATHOLOGICAL ANALYSIS

The STZ-induced DM rats showed the accumulation of lipid, indicating the increase in the concentration of lipid peroxidation products in their livers. Hepatic steatosis (formation of fat in the liver) was formed in the group of STZ induced DM rats (Figure 3). The BHE-treated rats' livers indicated an improvement of hepatic steatosis, which was in line with biochemical results showing the improved lipid profiles, especially in triglycerides levels.

The livers of BHE treated rats resembled the normal structure, and it was better than those of the metformin treated group which still showed some lipid droplets formation. Hyperlipidemia related with DM may be caused by enhanced hepatic triglyceride biosynthesis as well as the release of VLDL without an increase in the rate of clearance from the blood by lipoprotein lipase, which is dependent on the insulin/glucagon ratio (Adeneye et al. 2010). The normal rats' livers showed normal hepatic cells with well-preserved central vein, nucleus, and cytoplasm (Figure 3(A)). The untreated DM rats' livers had significant formation of lipid droplets, or fatty vacuoles, indicating fatty changes (Figure 3(B)). In BHE-treated rats' livers (Figure 3(C)) there was an improvement in hepatic cells which resembles normal structure of the cells (Figure 3(A)). The metformin-treated rats' livers (Figure 3(D)) showed a minimal improvement in fatty vacuoles compared to the control DM group. On the other organ, the normal rats' kidneys indicated the normal structure with intact architectures of Bowman's capsule and renal tubules (Figure 4(A)). The untreated DM rats' kidneys showed tubular hydropic degeneration (Figure 4(B)). Meanwhile, the BHE-treated rats' kidneys (Figure 4(C)) showed an improvement in kidneys, resembling the normal structure. On the other observation, the metformin-treated rats' kidneys (Figure 4(D)) showed hydropic changes in proximal convoluted tubules. The kidneys of STZ-induced DM rats showed the tubular hydropic degeneration and widening of Bowman's space (Figure 4).

Polyuria, or frequent urination, is a common feature of DM which may be associated with the widening of Bowman's space and increased glomerular filtration status in the DM rats' kidneys. The BHE-treated rats indicated an improvement with a near-normal renal structure. Similar hydropic changes of renal tubules in the kidneys of STZ-induced DM rats were also observed in a previous study (Wang et al. 2011). This feature may be due to the resulting osmotic diuresis from high glucose concentration. Thus, the present result indicated positive protective effects of BH in diabetic kidney by decreasing hydropic changes of renal tubules. This condition showed the nephroprotective effects of BHE in DM rats. A study also reported the nephroprotective effects of BH extracts in restoring degenerative changes of paracetamol induced nephrotoxicity in rats (Esmail & Ali 2019; Varghese et al. 2013) and in kidney of mercury poisoning rat's (Al-Snafi 2013; Mingyu et. al 1995).

This histopathological examination may be associated with the biochemical evaluation indicating

an improvement in the K level, which is a biomarker of renal function test in BHE-treated DM rats. The metformin-treated DM rats showed a higher serum K level compared to the other groups. In line with the biochemical evaluation, the histopathological observation of the metformin-treated DM rats showed significant hydropic changes of renal tubules compared to the control DM rats. This condition may be associated with hyperkalemia, which is the condition of a higher K level in blood. Hyperkalemia is caused by the lack of insulin in the body (Palmer 2010). Insufficient insulin secretion leads to hyperglycaemia. Hyperglycaemia causes the movement of water out from the cells. This situation generates efflux of K ion from the cell and influx of Na ion into the cells.

TABLE 1. The effects of BHE on biochemical parameters in severely diabetic rats in response to different treatments over eight weeks (n=6)

Parameter	Control normal	Control DM	BHE	Metformin
Lipid profile				
Total cholesterol (mmol/l)	1.77 ± 0.09	2.28 ± 0.09	2.08 ± 0.14	2.06 ± 0.30
Triglycerides (mmol/l)	0.90 ± 0.15	0.78 ± 0.09	$0.42 \pm 0.07^*$	$0.44 \pm 0.07^*$
LDL (mmol/l)	0.14 ± 0.04	0.46 ± 0.11	0.38 ± 0.09	0.36 ± 0.19
Liver function test (LFT)				
Total protein (g/l)	59.33 ± 1.33	52.50 ± 2.60	56.60 ± 1.21	51.20 ± 3.57
ALT (U/l)	37.33 ± 3.93	$75.50 \pm 0.87^*$	61.20 ± 4.38	$76.80 \pm 8.87^*$
ALP (U/l)	136.0 ± 4.16	$1246 \pm 99.79^*$	$729.8 \pm 116.10^{*,\#}$	$576.4 \pm 127.70^*$
Renal function test (RFT)				
Urea (mmol/l)	6.10 ± 0.60	$13.13 \pm 0.98^*$	12.04 ± 1.49	11.55 ± 2.17
Creatinine (umol/l)	52.80 ± 5.08	68.20 ± 2.20	54.56 ± 3.29	66.88 ± 7.15
Potassium (mmol/l)	6.30 ± 0.14	6.20 ± 0.33	5.4 ± 0.75	7.46 ± 0.98
Glycosylated haemoglobin				
HbA1c %	4.00 ± 0.21	$8.03 \pm 0.24^*$	$7.98 \pm 0.28^*$	$8.48 \pm 0.13^*$

*$p<0.05$ compared to control normal; #$p<0.05$ compared to control DM

Hydropic changes occur because the injured cells fail to maintain the equilibrium of electrolytes through the sodium-potassium pump. Renal changes in metformin-treated DM rats may be associated with the side effects of the drug. Lactic acidosis may occur in type 2 DM patients who are treated with metformin, and this occurrence can cause renal failure (Pertek et al. 2003). An early study reported that a type 2 DM patient

developed lactic acidosis and severe hyperkalemia after taking metformin and enalapril treatment (Franzetti et al. 1994). This may explain the histopathological changes in metformin-treated rats' kidneys (Figure 4).

LETHAL DOSAGE

No toxic effect was observed from the treatment with BHE of up to dose 2500 mg/kg and 3750 mg/kg up to 24 h. The behaviours of the treated animals were normal, and no mortality occurred in any of the experimental group. The food consumption and urine output were also normal. The assessment of the toxic action of the BHE is indispensable particularly to ensure the extract is safe. It enables us to recognize the intrinsic toxicity of the plant and the effects of acute overdose. The rats were observed for gross behavior, autonomic sign and neurology sign.

Bioactive compounds derived from plants can potentially reduce the BGL at the same time to minimize several diabetes complications, such as oxidative stress, kidney impairment, and fatty liver (Bahadoran et al. 2013). DM is commonly associated with increasing oxidative stress and decreasing anti-oxidative ability (Maxwell et al. 1997). Abnormalities are caused by oxidative stress, including renal tubular injury and gradual loss of renal function (Shah et al. 2007). BH contains wide range of bioactive compounds and one of that is gallic acid which has been reported to have antihyperglycaemic, antilipid peroxidative, and antioxidant effects on STZ-induced DM Wistar rats (Punithavathi et al. 2011a). The present result was supported by findings from another study which reported nephroprotective effects of the hydro-alcoholic extract of BH against paracetamol-induced nephrotoxicity (Varghese et al. 2013). The dose chosen in this study can be extrapolated to human dose or other wildlife using dose conversion method (Reagan-Shaw et al. 2008).
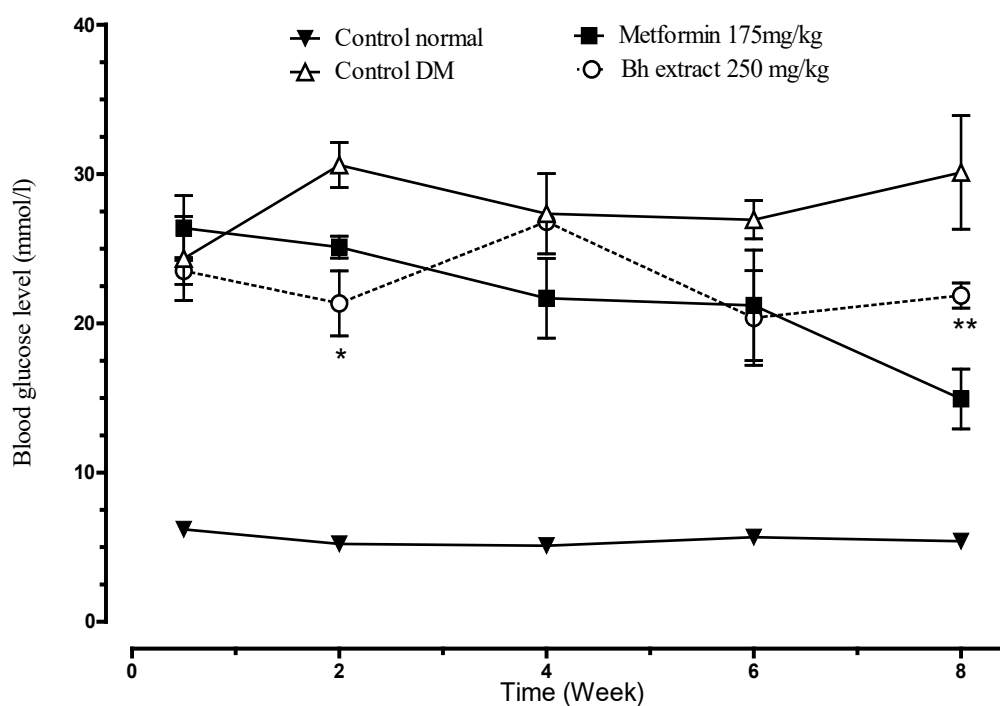

FIGURE 1. The blood glucose level of severely diabetic rats in response to BHE treatment within eight weeks (n=6)
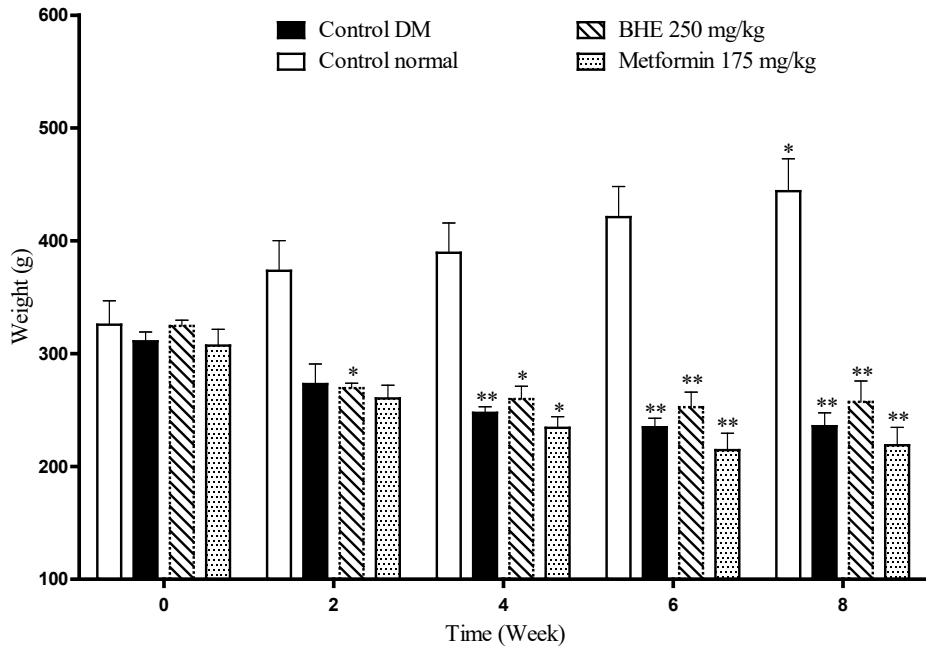
($^*p<0.01$ compared to DM, $^{**}p<0.05$ compared to DM)

FIGURE 2. Weight changes of severely diabetic rats in response to different treatments over eight weeks (n=6)

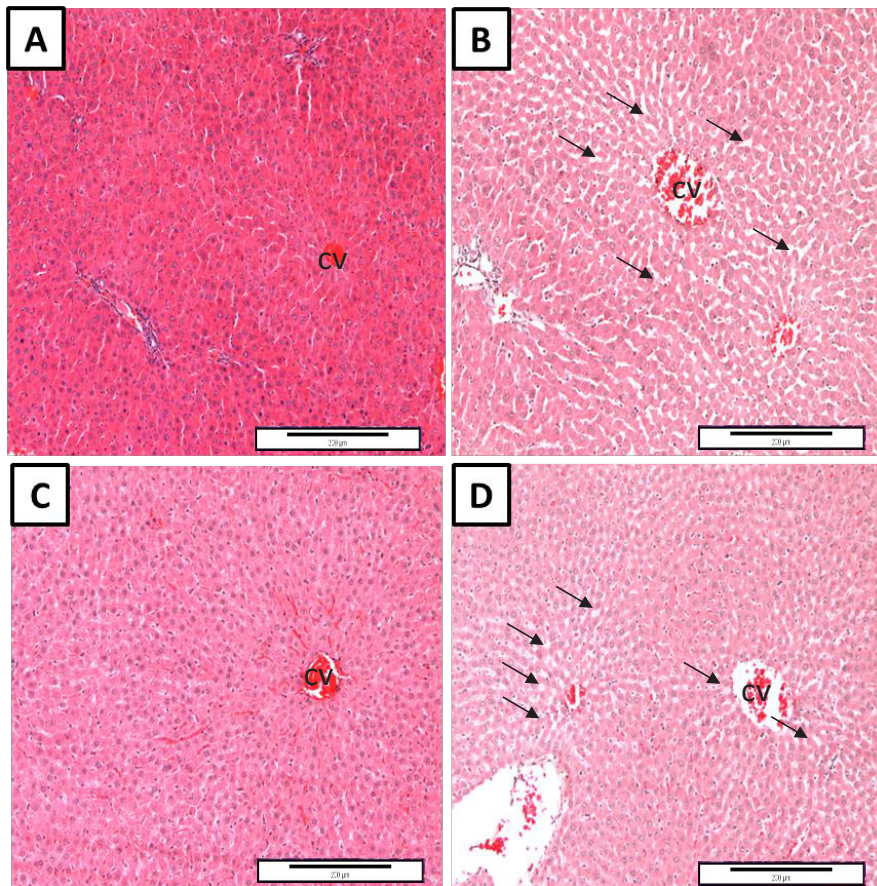*$p<0.05$; **$p<0.01$ compared to W0 in the respective group

FIGURE 3. Liver tissue slices from (A) normal rat, (B) DM rat, (C) BHE-treated rat, and (D) metformin-treated rat after eight weeks (All figures are in 10× magnification and 200 μm scale)

Arrow (→) showing white spots indicate formation of lipid droplets, CV= central vein
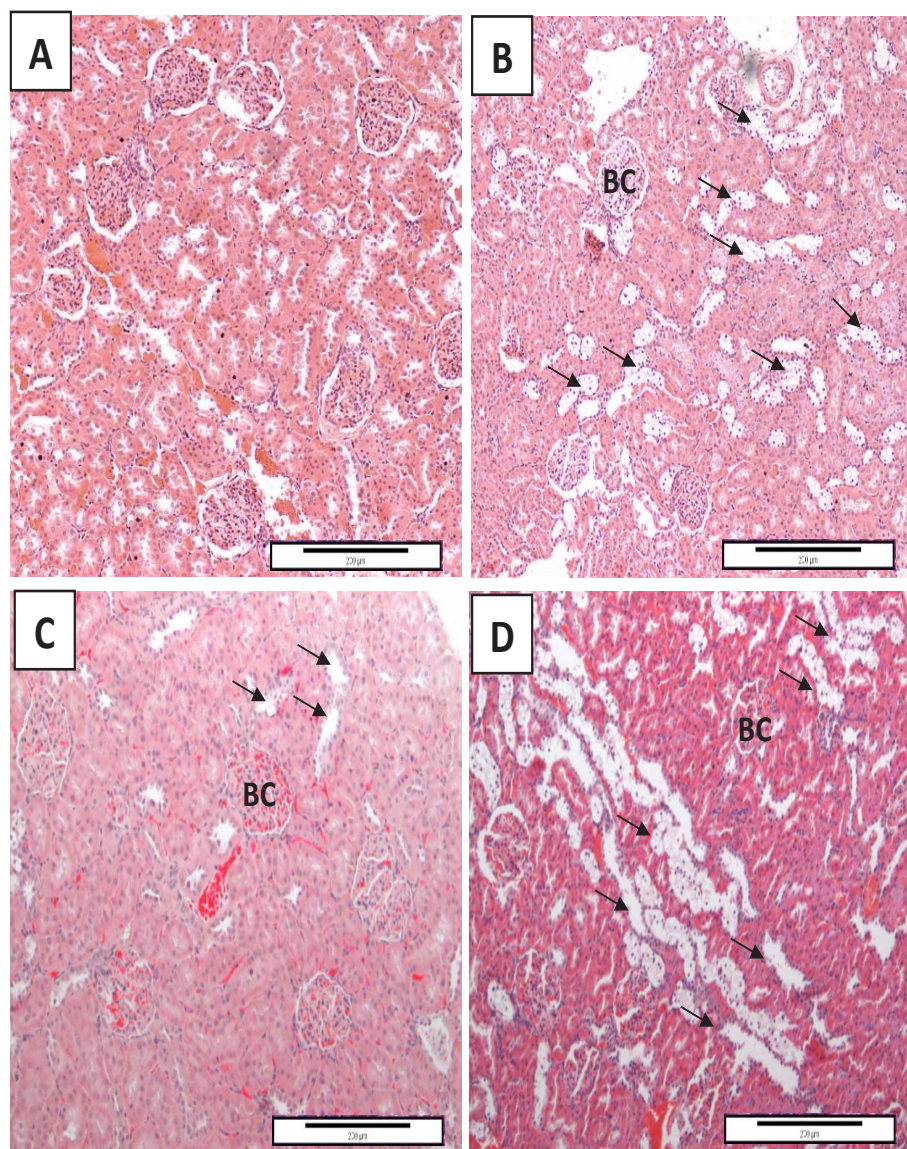
FIGURE 4. Kidney tissue slices from (A) normal rat, (B) DM rat, (C) BHE-treated rat, and (D) Metformin-treated rat after eight weeks (All figures are in 10× magnification and 200 µm scale)

Arrow (→) showing hydropic changes, BC= Bowman's capsule

CONCLUSION

It can be concluded that BH possesses hypoglycaemic and protective effects against DM. The effects were associated with naturally occurring polyphenol in BH. In addition, the administration of BHE for eight weeks with effective dose of 250 mg/kg showed significant reduction in the BGL as well as improvement in weight and biochemical parameters, and in histopathological observation of liver and kidneys of diabetic rats.

A high LD_{50} of the extract is safe as the rats acted normal with no sign of restlessness, drowsiness, piloerection and convulsion throughout the study period. It indicates nontoxic nature of BHE, and it is safe to be consumed even in large concentration. Furthermore, a toxicity study investigated the safety profile of 70% ethanolic extract of BH reported the extract is non-toxic and safe for long term oral consumption (Shakya et al. 2020). The study also reported

LD$_{50}$ value of the BH extract was more than 2000 mg/kg and it did not show any change in biochemical, haematological, and histological parameters.

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