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Full Length Article

Nephroprotective Effects of High Active Deproteinized Extract of Calf Blood in Diabetic Rats

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Abstract

Evaluating the renal protective effect of calf blood deproteinized extract (DECB) on diabetic rats and exploring its underlying mechanisms. Rats with similar body weight (n=40) were divided into 4 groups. The NC group (n=10) was the blank control group and the remaining 30 were intraperitoneally injected with Streptozotocin (65 mg/kg) for 1 w. After 1w, they were randomly divided into 3 groups (n=10): model (M), combination (MD, 105 mg/kg metformin/378 mg/kg DECB) and metformin (MMet, 105 mg/kg metformin). After 8w, Body weights were recorded; urinary microalbumin (UALB) and urinary creatinine (UCR) levels in the urine were measured, as well as blood sugar, high-density lipoprotein (HDL-C), low-density lipoprotein (TGLDL-C), blood urea nitrogen (BUN), creatinine (Cr), total cholesterol (TC), triglyceride (TG), uric acid (UA), glutathione (GSH), malondialdehyde (MDA) levels, superoxide dismutase (SOD) and glutathione peroxidase (GSH-PX) in the serum of mice was determined. Histopathological examination of the rat kidneys was performed; western blot was used to determine specific LC3II, Atg5 and p62/SQSTM1 expression. In comparison with NC rats, the M group exhibited lower body weights, the levels of HDL-C, GSH, SOD and GSH-PX activities were reduced, while fasting blood glucose, UAlb, UCr, Cr, UA, BUN, LDL-C, TC, TG and MDA amounts were elevated ($P < 0.05$); Furthermore, Histopathological observation of the kidney showed that compared with the NC group, the glomerular congestion and tubular edema of the M group were obvious. Compared with the M group, the renal tissue lesions of the rats in each administration group were significantly improved and the interstitial hyperplasia was not obvious. The protein expression of LC3II、Atg5 and p62/SQSTM1 was significantly decreased in MD and MMet groups compared with M group ($P < 0.05$). Thus, it is concluded that DECB and metformin combination reduces blood glucose and regulates blood lipids in diabetic rats, improving the autophagy activity of podocytes in the renal tissues by inhibiting reactive oxygen species, thus to play a protected role in the treatment of diabetic nephropathy. © 2020 Friends Science Publishers

Keywords: Deproteinized extract of calf blood; Diabetic nephropathy; Antioxidant; Autophagy

Introduction

The deproteinized extract of burdock blood (DECB) is rich in matter. Inorganic substances in DECB account for 70%, including trace elements and electrolytes. Organic substances account for 30%, including sugars, nucleic acids, low molecular proteins, lipids and sugars and their derivatives (Xu *et al.* 2018). The dry matter weight of DECB is 40 mg/mL and it contains potassium ion, sodium ion, chlorine ion, peptides, amino acids and glucose. The concentration of potassium ion, sodium ion, chlorine ion, peptides, amino acids and glucose in DECB is 0.48±0.26, 15.92±1.98, 1.79±0.21, 1.07±0.16 and 2.01±0.32 mg/mL respectively in DECB is 0.48±0.26, 15.92±1.98, 1.79±0.21, 1.07±0.16 and 2.01±0.32 mg/mL respectively (Xu *et al.* 2018). The main components of DECB are phosphoinositol oligosaccharides and small molecule activating peptides, which can promonceote cellular uptake and utilization of glucose and oxygen (independent of insulin) and provide high energy for cells. (Macheret and Khanenko 2002; Lv *et al.* 2010). In addition, research shows it enhances body metabolic reserve and prolongs cell survival (Luo *et al.* 2006; Li *et al.* 2007). however, whether it protects from diabetic renal diseases remains largely unclear. In this study, we have investigated the effects of combination of DECB and metformin on blood glucose and blood lipids in diabetic rats and explore the role of DECB in the treatment of diabetic nephropathy.

Materials and Methods

Materials

Healthy male Wistar rats (n=40), weighing 165–189 g, license number: SCXK (Ji) 20140003 (Changchun Yis Experimental Animal Technology Co., Ltd., China); The

DECB with high activity was produced in-house by our laboratory. The assessment kits for high-density lipoprotein (HDL-C License No: A112-1-1), low-density lipoprotein (LDL-C License No: A113-1-1), total cholesterol (TC License No: A111-1-1), triglyceride (TG License No: A110-1-1), blood urea nitrogen (BUN License No: C013-1-1), serum creatinine (SCr License No: C012-1-1), uric acid (UA License No: C1014-1-1), urinary microalbumin (UAlb License No: C1016-1-1), urinary creatinine (UCr License No: A099-1-1) and superoxide dismutase (SOD License No: A001-3-2) were all manufactured by Biosino Bio-Technology and Science Inc. The assay kits for glutathione GHS peroxidase (GSH-PX License No: A005-1-2) and malondialdehyde (MDA License No: A003-1-2) detection were provided by Nanjing Jiancheng Bioengineering Institute. Hematoxylin-Eosin/HE Staining Kit (G1120). Rabbit polyclonal antibodiesLC3II (License No: KF446; Jackson ImmunoResearch)、Atg5 (License No: KF435; Jackson ImmunoResearch) and p62/SQSTM1 (License No: KF430; Jackson ImmunoResearch), 1: 800; Cell Signaling Technology) and actin (License No: KL002; Santa Cruz Biotechnology). Goat Anti-rabbit IgG Horseradish Peroxidase Conjugate (License No: KS002; Jackson ImmunoResearch). The biochemical incubator SPX-250B-Z (Shanghai Boyuan Industry Co., Ltd.), refrigerated centrifuge 5430R (Eppendorf, USA), UV spectrophotometer (Shimadzu) and Infinite M200 microplate reader (Tecan, Swiss) were employed in the current study.

Preparation of high active deproteinized extract of calf blood

Under sterile conditions, venous blood from calves at the age of 1–6 months was mixed and heated for sterilization. After sedimentation, the blood supernatant was filtered through an inorganic membrane (ceramic membrane 7) and hydrochloric acid was added to the filtrate to adjust the pH to 3–4. The resulting solution was submitted to ultrafiltration (20000 Dalton) and the pH of the collected filtrate was adjusted to 8–10. Then, the solution further underwent ultrafiltration (20000 Dalton) and the filtrate was collected and brought to neutral pH. The obtained solution was concentrated with a reverse osmosis membrane and submitted to ultrafiltration for the removal of proteins with more than 5000 Dalton to yield DECB. The final extract was lyophilized and the resulting powder was quantitatively dissolved to prepare the solution used for intragastric administration.

Establishment of the rat diabetes model

Sixty male Wistar rats were subjected to adaptive feeding for one week. Of these, 10 rats were randomly assigned to the normal control (NC) group and provided normal diet. Meanwhile, the remaining rats were intraperitoneally administered streptozotocin (STZ, 65 mg/kg) in 0.1 mol/L citrate buffer (pH 4.2) for diabetic model establishment. After one week, fasting blood glucose was measured, with a value $\geqslant$7.8 mmol/L selected as the criterion for successful modeling.

Animal grouping and drug administration

The successful diabetic rats with blood sugar levels of 7.8–16.0 mmol/L were randomly divided into 3 groups: model group (M group), combined drug group (MD group) and metformin group (MMEt group), 10 rats in each group. The MMet group was administered metformin intragastrically at 105 mg/kg. The MD group was administered DECB at 378 mg/kg intraperitoneally together with intragastric metformin (105 mg/kg). The rats were administered the drugs once daily for eight consecutive weeks.

Determination of biochemical indicators

After the administration period of eight weeks, rats were individually placed in metabolic cages to collect urine samples for 12 h. The supernatants were sampled for UAlb and UCr level determination. Then, the treated rats were anesthetized with 100 mg/kg urethane injected intraperitoneally for the collection of 4–5 mL blood from the abdominal aorta, followed by euthanasia. The obtained blood specimens were submitted to centrifugation (3500 rpm, 10 min) for serum preparation. The levels of various serum parameters like blood glucose, TC, TG, HDL-C, LDL-C, UA, BUN, Cr and MDA, as well as SOD, GSH and GSH-PX activities were assessed as directed by the manufacturers of specific kits.

Pathological examination of the kidney tissue

The rat kidney was extracted, submitted to fixation with 10% buffered formaldehyde and paraffin embedding, sectioned and incubated in presence of hematoxylin and eosin (H&E) for staining. The histopathological changes in the kidney were evaluated under a light microscope.

Western blot analysis

Static digestion and discontinuous gradient centrifugation were used to separate rat kidneys. Cut the frozen rat kidneys into pieces, and incubated with type II collagenase and Hank's solution for 15 min. After digestion, rats' kidneys were washed with Hanks' solution. Preparation of Rat Kidney Homogenate at 4℃. The protein levels of LC3II, ATG5 and p62/sqstm1 were determined by Western blotting. The 80 μg protein samples were electrophoretized by 10% SDS-PAGE, and then transfected by polyvinylidene difluoride (PVDF) membrane (Bio-Rad). The blots were detected with rabbit polyclonal antibody and incubated with horseradish peroxidase-binding secondary antibody. Protein

bands were radiographed by enhanced chemical energy spectroscopy. The protein bands were scanned by imaging densitometer and quantified by image analysis software.

Statistical analysis

All values were expressed as mean ± standard deviation (mean ± S). 19.0 SPSS software was used for the statistical analysis. $P < 0.05$ were considered significant differences statistically.

Results

Rat blood glucose levels and body weights

All groups showed similar body weights before diabetic model establishment. After modeling, the M group had markedly lower body weights and starkly higher blood glucose amounts in comparison with NC group ($P < 0.05$). In comparison with the M group, the MD and MMet groups displayed significantly enhanced body weights, while blood glucose was remarkably decreased ($P < 0.05$). These results are summarized in Table 1 and Fig. 1 and 2.

Detection of biochemical indicators

In comparison with NC group the M group showed significantly increased levels of UAlb and UCr ($P < 0.05$). The levels of UAlb and UCr amounts in the MD group were markedly decreased in comparison with NC group rats ($P < 0.05$). Additionally, UAlb and UCr amounts were starkly reduced in the MD group than in MMEt group ($P < 0.05$). These results are shown in Table 2 and Fig. 3 and 4.

In comparison with NC rats, the MD group showed starkly increased serum Cr, UA and BUN amounts ($P < 0.05$). However, serum Cr, UA and BUN were markedly decreased in the drug-combination group ($P<0.05$). Furthermore, serum Cr, UA and BUN in the MD group showed significant reductions ($P < 0.05$) in comparison with the MMet group. These results are shown in Table 3 and Fig. 5 and 6.

It was found that serum LDL-C, TC and TG amounts in NC group rats were markedly increased ($P < 0.05$) in comparison with NC values, whereas HDL-C was significantly decreased ($P < 0.05$). Interestingly, LDL-C and TC amounts in the MD group showed significant reductions, with HDL-C starkly increasing ($P < 0.05$) in comparison with NC group rats, but not significantly different from the values of the MMet group. Serum TG amounts in the MD and MMet groups were both markedly reduced ($P < 0.05$). These results are shown in Table 4 and Fig. 7 and 8.

The activities of serum SOD and GSH-PX, and GSH amounts in untreated models showed significant reductions ($P < 0.05$) in comparison with NC values. Serum SOD and GSH-PX activity levels, as well as GSH amounts were significantly elevated in the MD and MMet groups ($P < 0.05$) than in NC group rats. In addition, serum MDA levels were significantly elevated ($P < 0.05$) in the M group than in NC rats. Serum MDA levels in the MD and MMet groups were starkly reduced ($P < 0.05$) in comparison with the M group. These results are shown in Table 5 and Fig. 9, 10, 11 and 12.

Histological changes of the kidney tissue in rats

The size and morphology of renal tubules and glomeruli were normal in the NC group, with thin and clear glomerular blood vessels. Glomeruli in the M group were comparatively hyperemic and ruptured, with renal tubules severely edematous; in addition, there was mild interstitial hyperplasia in H&E staining. In the MMet group, hyperemia was much severe. The MD group exhibited significant improvement compared with the M group, with no significant interstitial hyperplasia or increased glomeruli, indicating that DECB may improve kidney tissue damage (Fig. 13).

The expression of LC3II, Atg5 and p62/SQSTM1 in the glomerular tissue of rats

Compared with the normal control group, the expression of LC3II, Atg5 and p62/SQSTM1 in the glomerular tissue of rats in the M group was significantly increased ($P < 0.01$). Compared with the M group, the expression of LC3-II, Atg5 and p62/SQSTM1in the glomerular tissue of rats in the DECB combined with metformin treatment group was significantly decreased ($P < 0.05$) (Fig. 14)

Discussion

Diabetic nephropathy (DN) is a major complication of diabetic microangiopathy and is observed in 20 to 40% of diabetic patients (Satirapoj and Adler 2014; Chen *et al.* 2017; Yu and Boventre, 2018). In this study, the protective effects of DECB on STZ-induced diabetic rats was investigated. The STZ-induced diabetic model was established after intragastric administration of DECB for eight weeks. The effect of DECB combined with metformin on DN rats was then evaluated for its protective effects. Elevated UAlb and UCr levels are signs of vascular systematic changes and are the early indicators of renal and cardiovascular dysfunction (Brenner *et al.* 2011; Zhang *et al.* 2018). After eight weeks of treatment, UAlb and UCr levels in the M group were increased, suggesting damage to the kidneys in diabetic rats. Pathological changes were evident and included glomerular hyperemia and tubular edema in the kidney tissues in rats in the M group. Serological tests demonstrated significantly increased serum BUN, UA and Cr levels in the M group rats and were due to decreased glomerular filtration rates. After treatment with DECB combined with metformin, serum levels of BUN, UA and Cr were dramatically reduced. This clearly demonstrated the

Table 1: Changes of blood glucose levels and body weights in different groups

Group	n	Weight (g)	Blood glucose (mmol/L)
NC	10	285.45 ± 14.34	4.71 ± 0.41
M	10	115.47 ± 31.27*	28.80 ± 3.65*
MD	10	146.83 ± 23.53△	22.62 ± 4.91△
MMet	10	133.45 ± 25.94△	27.05 ± 3.53

*P < 0.05 *vs.* NC; △P < 0.05 *vs.* M
NC: normal rats group; M: diabetic group; MD: combination group (105 mg/kg metformin/378 mg/kg DECB); MMet: metformin group (105 mg/kg metformin)

Table 2: UAlb and UCr contents in different groups

Group	n	UAlb (mg)	UCr (μmol/L)
NC	10	19.96 ± 1.52	20.86 ± 2.14
M	10	41.94 ± 3.22*	35.29 ± 7.38*
MD	10	34.53 ± 1.98△#	28.85 ± 6.73△#
MMet	10	39.72 ± 2.17	34.59 ± 6.92

*P < 0.05 *vs.* NC; △P < 0.05 *vs.* M; #P < 0.05 *vs.* MMet
NC: normal rats group; M: diabetic group; MD: combination group (105 mg/kg metformin/378 mg/kg DECB); MMet: metformin group (105 mg/kg metformin)

Table 3: Serum levels of Cr, UA and BUN in various treatment groups

Group	n	SCr (mmol/L)	UA (μmol/L)	BUN (mmol/L)
NC	10	7.46 ± 1.64	456.01 ± 78.25	6.52 ± 0.15
M	10	17.24 ± 3.41*	771.57 ± 70.79*	13.45 ± 0.40*
MD	10	12.65 ± 3.14△#	693.29 ± 57.33△#	8.88 ± 1.63△#
MMet	10	17.09 ± 3.22	769.81 ± 14.04	13.24 ± 0.78

*P < 0.05 *vs.* NC; △P < 0.05 *vs.* M; #P < 0.05 *vs.* MMet
NC: normal rats group; M: diabetic group; MD: combination group (105 mg/kg metformin/378 mg/kg DECB); MMet: metformin group (105 mg/kg metformin)

Table 4: Serum LDL-C, HDL-C, TC and TG in different groups

Groups	n	LDL-C (mmol/L)	HDL-C (mmol/L)	TC (mmol/L)	TG (mmol/L)
NC	10	1.75 ± 0.08	1.22 ± 0.09	1.03 ± 0.07	1.03 ± 0.07
M	10	3.43 ± 0.08*	0.61 ± 0.06*	15.61 ± 0.53*	1.81 ± 0.29*
MD	10	3.02 ± 0.53△	0.73 ± 0.09△	13.71± 1.58△	1.41 ± 1.13△
MMet	10	3.26 ± 0.18	0.65 ± 0.03	14.57 ± 1.13	1.46 ± 0.23△

*P < 0.05 *vs.* NC; △P < 0.05 *vs.* M
NC: normal rats group; M: diabetic group; MD: combination group (105 mg/kg metformin/378 mg/kg DECB); MMet: metformin group (105 mg/kg metformin)

Table 5: Serum SOD, GSH-PX, MDA and GSH amounts in different groups

Group	n	SOD (U/mg prot)	GSH-PX (U/mg prot)	MDA (μmol/mg prot)	GSH (μmol/mg prot)
NC	10	439.45 ± 20.46	99.02 ± 9.50	2.44 ± 0.24	463 ± 25
M	8	314.83 ± 8.51*	14.99 ± 2.15*	3.50 ± 0.24*	311 ± 56*
MD	8	333.43±14.53△	26.23 ± 8.57△	3.14 ± 0.33△	364 ± 49△
MMet	8	325.32 ± 8.57△	18.67 ± 5.12△	3.29 ± 0.22△	333 ± 31△

*P < 0.05 *vs.* NC; △P < 0.05 *vs.* M
NC: normal rats group; M: diabetic group; MD: combination group (105 mg/kg metformin/378 mg/kg DECB); MMet: metformin group (105 mg/kg metformin)

protective effects of DECB on glomerular filtration function. The underlying mechanism may be attributed to the phosphoinositide oligosaccharides and small molecular activator peptides found in DECB. The latter induces the mitochondria to synthesize ATP, improve the cellular utilization of oxygen during ischemic conditions, activate cells and switches anaerobic glycolysis to aerobic

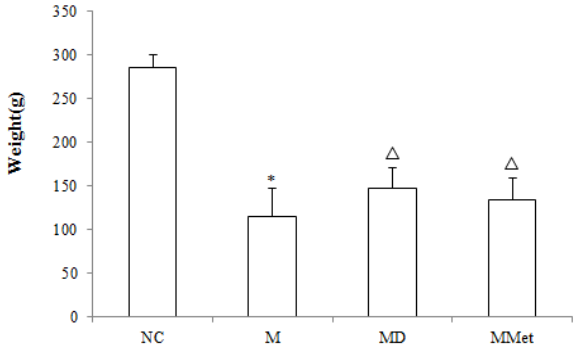

Fig. 1: Body weight changes in different groups
*P < 0.05 *vs.* NC; △P < 0.05 *vs.* M
NC: normal rats group; M: diabetic group; MD: combination group (105 mg/kg metformin/378 mg/kg DECB); MMet: metformin group (105 mg/kg metformin)

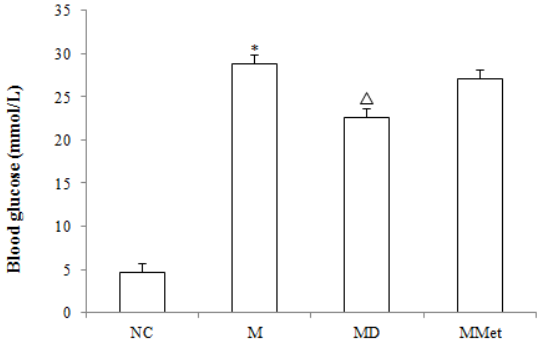

Fig. 2: Changes of blood glucose levels in different groups
*P < 0.05 *vs.* NC; △P < 0.05 *vs.* M
NC: normal rats group; M: diabetic group; MD: combination group (105 mg/kg metformin/378 mg/kg DECB); MMet: metformin group (105 mg/kg metformin)

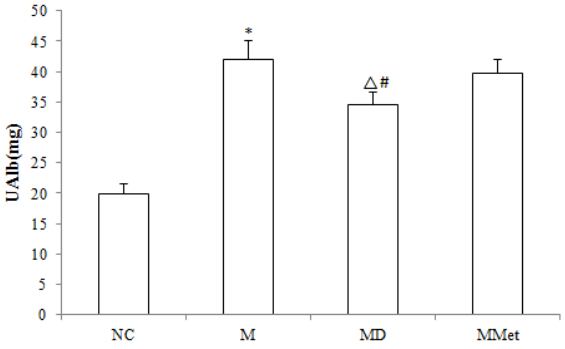

Fig. 3: UAlb contents in different groups
*P < 0.05 *vs.* NC; △P < 0.05 *vs.* M; #P < 0.05 *vs.* MMet
NC: normal rats group; M: diabetic group; MD: combination group (105 mg/kg metformin/378 mg/kg DECB); MMet: metformin group (105 mg/kg metformin)

carbohydrate metabolism in cells. All this suggests that DECB may prolong cell survival under hypoxic conditions (Wang *et al.* 2015) and improve tissue immune defense. In addition, DECB has been shown to inhibit nitric oxide synthesis, which is an important mediator during the ischemic cascade. Hence, DECB could block the ischemic cascade to improve renal ischemia and glomerular filtration to retain renal function (Schuelert *et al.* 2015).

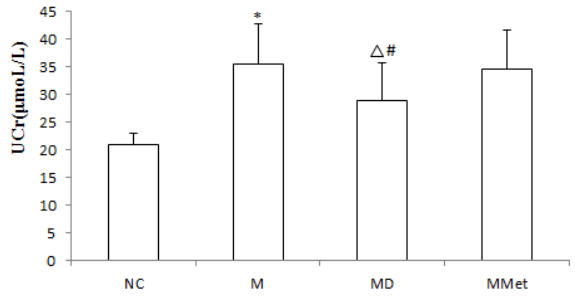

Fig. 4: UCr contents in different groups
*$P < 0.05$ *vs.* NC; $^{\triangle}P < 0.05$ *vs.* M; $^{\#}P < 0.05$ *vs.* MMet
NC: normal rats group; M: diabetic group; MD: combination group (105 mg/kg metformin/378 mg/kg DECB); MMet: metformin group (105 mg/kg metformin)

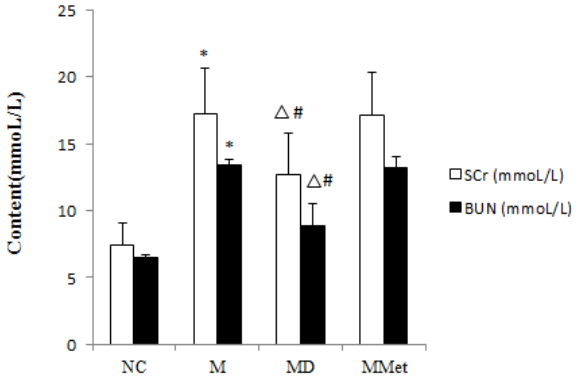

Fig. 5: Serum levels of Cr and BUN in different groups
*$P < 0.05$ *vs.* NC; $^{\triangle}P < 0.05$ *vs.* M; $^{\#}P < 0.05$ *vs.* MMet
NC: normal rats group; M: diabetic group; MD: combination group (105 mg/kg metformin/378 mg/kg DECB); MMet: metformin group (105 mg/kg metformin)

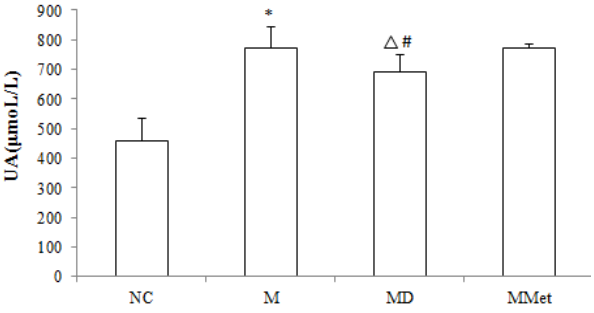

Fig. 6: Serum levels of UA in different groups
*$P < 0.05$ *vs.* NC; $^{\triangle}P < 0.05$ *vs.* M; $^{\#}P < 0.05$ *vs.* MMet
NC: normal rats group; M: diabetic group; MD: combination group (105 mg/kg metformin/378 mg/kg DECB); MMet: metformin group (105 mg/kg metformin)

In addition, co-administration of DECB and metformin could reduce hyperlipidemia in diabetic rats. LDL-C levels in both the treatment groups were significantly lower, while HDL-C levels were significantly increased after co-administration of DECB and metformin to diabetic rats. TC and TG levels in the M group were markedly increased, indicating the development of vascular lesions (Tan *et al.* 2014). After co-administration of DECB and metformin, TC and TG levels were significantly

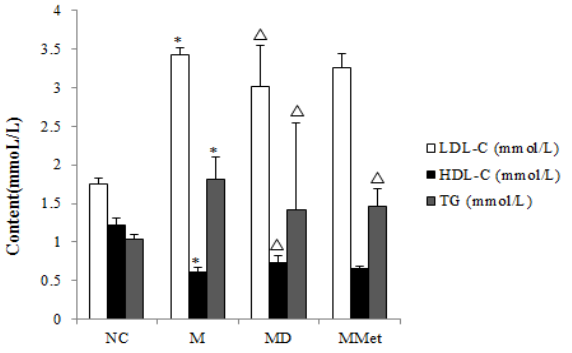

Fig. 7: Serum LDL-C, HDL-C and TG in different groups
*$P < 0.05$ *vs.* NC; $^{\triangle}P < 0.05$ *vs.* M
NC: normal rats group; M: diabetic group; MD: combination group (105 mg/kg metformin/378 mg/kg DECB); MMet: metformin group (105 mg/kg metformin)

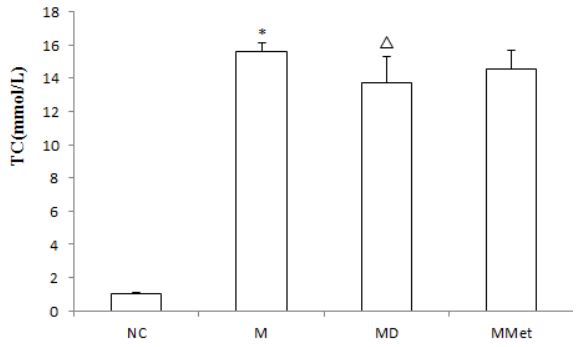

Fig. 8: Serum TC in different groups
*$P < 0.05$ *vs.* NC; $^{\triangle}P < 0.05$ *vs.* M
NC: normal rats group; M: diabetic group; MD: combination group (105 mg/kg metformin/378 mg/kg DECB); MMet: metformin group (105 mg/kg metformin)

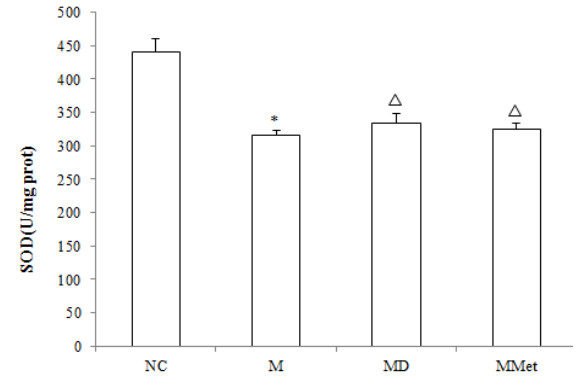

Fig. 9: Serum SOD levels in different groups
*$P < 0.05$ *vs.* NC; $^{\triangle}P < 0.05$ *vs.* M
NC: normal rats group; M: diabetic group; MD: combination group (105 mg/kg metformin/378 mg/kg DECB); MMet: metformin group (105 mg/kg metformin)

decreased, reflecting the protective effects of DECB on diabetic vascular lesions. Diabetic rats undergone increased oxidative stress and have significantly reduced levels of SOD, GSH and GSH-PX, and significantly increased levels of MDA (Hartnett *et al.* 2000). DECB could significantly increase serum SOD, GSH and GSH-PX levels in diabetic

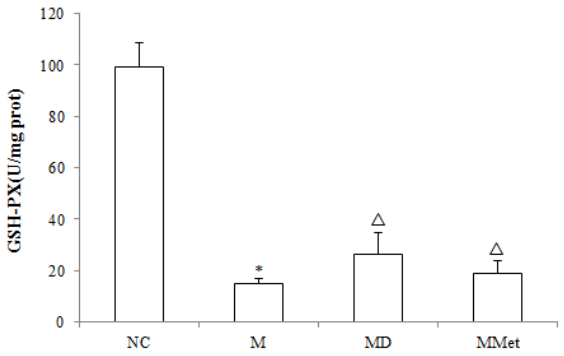

Fig. 10: Serum GSH-PX levels in different groups
*$P < 0.05$ *vs.* NC; △$P < 0.05$ *vs.* M
NC: normal rats group; M: diabetic group; MD: combination group (105 mg/kg metformin/378 mg/kg DECB); MMet: metformin group (105 mg/kg metformin)

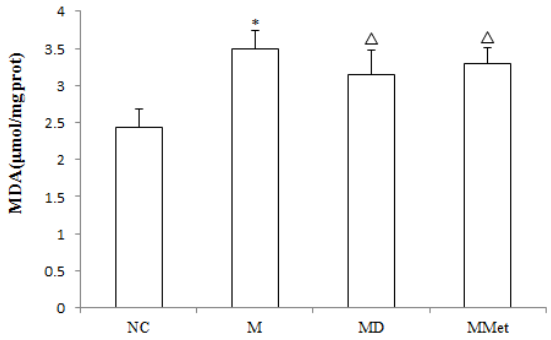

Fig. 11: Serum MDA levels in different groups
*$P < 0.05$ *vs.* NC; △$P < 0.05$ *vs.* M
NC: normal rats group; M: diabetic group; MD: combination group (105 mg/kg metformin/378 mg/kg DECB); MMet: metformin group (105 mg/kg metformin)

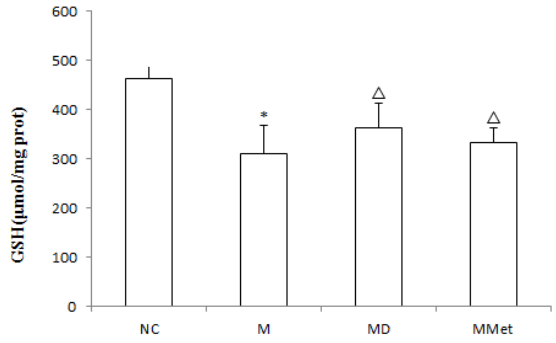

Fig. 12: Serum GSH levels in different groups
*$P < 0.05$ *vs.* NC; △$P < 0.05$ *vs.* M
NC: normal rats group; M: diabetic group; MD: combination group (105 mg/kg metformin/378 mg/kg DECB); MMet: metformin group (105 mg/kg metformin)

rats, while simultaneously decreasing MDA levels. This strongly suggests that DECB may protect the kidney from injury by reducing oxidative stress. Histopathological examinations demonstrated significant improvement in kidney lesions as well as reduced vacuolar degeneration in the renal tubules. No significant interstitial hyperplasia was observed in the MD and MMet groups compared to model rats in the M group.

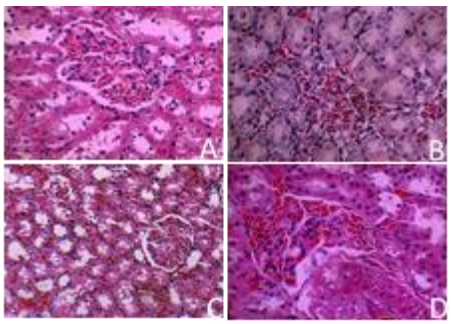

Fig. 13: Histology of the rat kidney stained with H&E (×400) [(**A**) Control; (**B**) Model; (**C**) MD; (**D**) MMet
NC: normal rats group; M: diabetic group; MD: combination group (105 mg/kg metformin/378 mg/kg DECB); MMet: metformin group (105 mg/kg metformin)

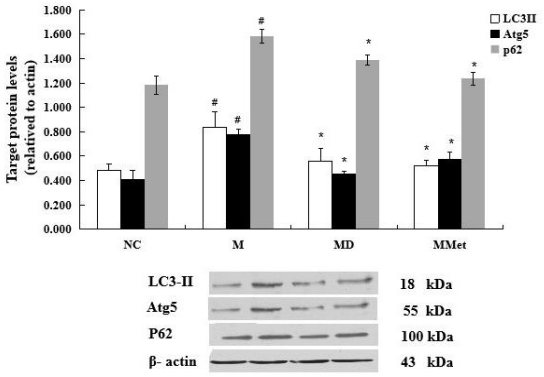

Fig. 14: The expression of LC3II, Atg5 and p62/SQSTM1 in the glomerular tissue of rats
#$P < 0.05$ *vs.* NC; *$P < 0.05$ *vs.* M
NC: normal rats group; M: diabetic group; MD: combination group (105 mg/kg metformin/378 mg/kg DECB); MMet: metformin group (105 mg/kg metformin)

Autophagy maintains podocyte homeostasis. Podocytes are an important component of the glomerular basement membrane. They are terminally differentiated cells and hence lack the ability to regenerate. This is one of the reasons that limits the repair to renal function. Hence, podocyte injury plays an important role during glomerular diseases (Lin *et al.* 2019). Under normal physiological conditions, basal autophagy levels in podocytes are relatively high. However, during diabetic nephropathy, podocytes are continuously exposed to oxidative stress or DNA damage due to persistent high glucose levels and increased local ROS in renal tissues. Podocytes are unable to eliminate excess damaged DNA generated during DNA synthesis as they are terminally differentiated. They solely rely on autophagosomes to remove damaged proteins and organelles (Yang *et al.* 2018). The expression levels of autophagy-related proteins LC3-II, Atg5 and p62/SQSTM1 in glomerular tissues in rats with diabetic nephropathy are increased. After administration of DECB, the expression levels of autophagy-related proteins were decreased in the treatment group. This suggests that DECB could improve autophagy in podocytes.

Our study demonstrated that DECB co-administered

with metformin could decrease blood glucose levels in diabetic rats and improve in renal pathology by lowering UAlb and UCr levels. We demonstrated that DECB could substantially reduce kidney damage. In addition, DECB reduced serum TC, TG and LDL-C levels and increased HDL-C levels to regulate blood lipids. Furthermore, co-administration of DECN and metformin increased SOD, GSH-PX and GSH levels while simultaneously reducing MDA levels, to enhance antioxidant capacity. Finally, the combination of DECB and metformin reduced blood glucose levels, regulated blood lipids in diabetic rats and improved autophagy in podocytes by inhibiting ROS. Hence, DECB plays a vital role in the treatment of diabetic nephropathy.

Conclusion

A combination of DECB and metformin reduces blood glucose levels, regulates blood lipids in diabetic rats, and improves autophagy in podocytes by inhibiting ROS. All this suggests that DECB plays a vital role in the treatment of diabetic nephropathy.

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