Print ISSN: 2319-2003 | Online ISSN: 2279-0780

IJBCP International Journal of Basic & Clinical Pharmacology

DOI: http://dx.doi.org/10.18203/2319-2003.ijbcp20190668

Original Research Article

DPP-4 inhibitory activity and myocardial salvaging effects of *Commiphora mukul* in experimental diabetes

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Received: 07 January 2019
Accepted: 29 January 2019

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ABSTRACT

Background: *Commiphora mukul* (Burseraceae) is commonly known as Guggul in Ayurveda. Several studies have reported antidiabetic activity of *Commiphora mukul* but there are no studies to explore the DPP-4 inhibitory activity and myocardial salvaging effects of *Commiphora mukul* in setting of diabetes mellitus. The present study was designed to evaluate the cardioprotective efficacy as well as safety of the medicinal plant *Commiphora mukul* (Guggul) in the experimental model of myocardial infarction co-existing with diabetes.
Methods: Diabetes was induced with single dose of streptozotocin (STZ): 45mg/kg ip and myocardial infarction was produced by administering isoproterenol (ISP): (85mg/kg, sc) to rats 24 and 48 h prior to scarification (5th week). After the confirmation of diabetes on 7th day (glucose>200mg/dl), vildagliptin (10 mg/kg) and *Commiphora mukul* (200 mg/kg) were administered orally from 1st to 5th week (4 weeks). At the end of experimental period, normal control, diabetic-isoproterenol control, vildagliptin and *Commiphora mukul* group rats were sacrificed for further biochemical investigations as well as histopathological evaluation.
Results: *Commiphora mukul* treatment demonstrated significant antidiabetic as well as myocardial salvaging effects as indicated by restoration of blood glucose, HbA1c and CPK-MB serum DPP-4, hs-CRP levels as compared to diabetic ISP control group. In addition, *Commiphora mukul* showed significant cardioprotection as indicated by positive correlation between cardiac marker CPK-MB and serum DPP-4. The histopathological assessment of heart, pancreas and biochemical indices of injury confirmed the cardioprotective effects of *Commiphora mukul*. In addition, *Commiphora mukul* was found to be safe to the liver and kidney.
Conclusions: The natural DPP-4 inhibitor *Commiphora mukul* demonstrated significant cardioprotective effects in experimental model of myocardial infarction co-existing with diabetes.

Keywords: *Commiphora mukul*, DPP-4 inhibitor, Diabetes, Experimental model, Myocardial infarction

INTRODUCTION

A large body of epidemiological and pathological evidence documents that type 2 diabetes mellitus (T2DM) is an independent risk factor for cardiovascular disease (CVD) and is associated with increased susceptibility to cardiovascular complications.[1,2] Cardiovascular complications are associated with increased mortality and morbidity related with diabetes mellitus. To make matters worse, when patients with diabetes develop clinical CVD, they sustain worse prognosis for survival than patients of CVD without diabetes.[3-5] These statistics provides scientific rationale for aggressively addressing the cardiovascular complications of diabetes. Therefore, the therapeutic goal, besides glycemic control in management

of diabetes, should also be to improve the adverse cardiovascular outcomes in diabetic patients.

All the major classes of oral anti-diabetes medications reduce hyperglycemia, although through different mechanisms.[6] Reduction of hyperglycemia decreases the risk for microvascular complications, in patients with T2DM.[7] Nonetheless, a large pool of patients with diabetes dies of macrovascular disease such as CVD. Therefore, an understanding of the cardiovascular effects of various oral antidiabetic medications is necessary to address the cardiovascular complications of diabetes. Anti-diabetic drugs, which can lower blood glucose levels as well as lower the risk for cardiovascular events are ideal because they treat both disorders which usually co-exist.

Several new synthetic drugs with glucose-lowering efficacy offering specific advantages have recently become available for clinical use. Since 2005, new incretin based drugs have been approved. These include injectable Glucagon-like peptide 1 (GLP-1) receptor agonists and oral dipeptidyl peptidase-4 (DPP-4) inhibitors.[8] Native GLP-1 has a short half-life of minutes, being rapidly degraded by DPP-4, to generate an NH2-terminally truncated metabolite in addition to undergoing renal excretion. DPP-4 inhibitors prolong the circulating half-life of GLP-1 and may therefore afford potential cardiovascular beneficial effects. Besides being established as standards antidiabetic effects various preclinical data and mechanistic studies have reported the cardioprotective effects of DPP-4 inhibitors, via GLP and GLP-1 dependent and independent mechanisms.[9] Studies documented that, DPP-4 inhibitors improve several cardiovascular risk factors: they improve glucose control (by reducing the risk of post prandial and fasting hyperglycemia), are weight neutral, lower blood pressure, improve dyslipemia, reduce inflammatory markers, diminish oxidative stress, improve endothelial functions and reduce platelet aggregation in patients with T2DM [10,11] Since a large pool of diabetic patients has cardiovascular co-morbidites, DPP-4 inhibitors may therefore represent promising oral hypoglycemic agents beneficial in this subset of diabetic patients with cardiovascular diseases.

Commiphora mukul is commonly known as Guggul in Ayurveda. It belongs to family Burseraceae, has been used to treat various conditions such as obesity, inflammation, hyperlipidemia, diabetes and diabetic cardiomyopathy.[12] Although the anti-diabetic, cardioprotective activity of *Commiphora mukul* has been reported, its ability to alter the DPP-4 pathway in experimental models had not been studied so far. In this scenario, research to identify novel DPP-4 inhibitors that favorably modify various CVD risk factors, work in concert with the body's own defenses, are less expensive and have fewer side effects, are desirable. With this point of view, this study was designed to identify medicinal plants with DPP-4 inhibitory activity which could be further developed as natural alternatives to the synthetic DPP-4 inhibitors. The cardioprotective efficacy as well as safety of the medicinal plant *Commiphora mukul* (Guggul) was evaluated in the experimental model of myocardial infarction co-existing with diabetes.

METHODS

Chemicals and test drugs

Streptozotocin (STZ) and Isoproterenol (ISP) were procured from Sigma Chemicals St Louis, USA. The test drugs Vildagliptin (Galvus tab.) was obtained as gift sample from Novartis pharmaceuticals UK Ltd. The hydro-alcoholic dried standardized extract of *Commiphora mukul* (Gugglu) were procured from Sanat Pharmaceutical, New Delhi. All other chemicals and reagents used were of analytical grade.

Experimental animals

Experimental study was conducted at Mahatma Gandhi Mission Medical College, Navi Mumbai, India for a period of one year from month of March 2015 to March 2016. Wistar rats were procured from Bombay Veterinary College, BVC Campus Road, Parel, Mumbai. Adult male Wistar rats (Normal Control [8], Diabetic ISP Control [13], Vildagliptin [10] and *Commiphora mukul* [10], 10 to 12 weeks old, weighing 150 to 200 gm were used in the study. Rats were housed in the Animal Facility of Mahatma Gandhi Mission Medical College, Navi Mumbai, India in polyacrylic cages (38x23x10cm) under standard laboratory conditions. The animals were allowed free excess to standard diet, tap water *adlibitum* and allowed to acclimatize for one week before the experiments. The study protocol was approved by the Institutional Animal Ethics Committee and conforms to the *Committee for the Purpose of Control and Supervision of Experiments on Animals* and Indian National Science Academy and Guidelines for the Use and Care of Experimental Animals in Research.

Experimental model of myocardial infarction in the setting of diabetes mellitus

Male Wistar rats weighing 150- 200gm was used for the study. A pilot study was carried out with different doses of STZ in order to select the appropriate dose of STZ for induction of type 2 diabetes mellitus. Based on the pilot study results, it was found that 45mg/kg STZ produced partial destruction of pancreatic cells resulting in type 2 diabetes mellitus in experimental rats. Therefore, a single Streptozotocin injection (45mg/kg body wt, intraperitoneal {i.p.} dissolved in 0.01M cold citrate buffer, pH 4.5) was standardized for induction of type 2 diabetes mellitus in overnight fasted rats. Serum glucose estimations (blood sugar >200mg/dL) were undertaken periodically (day 0, 3 and 7) from the tail vein to confirm the production of diabetes mellitus. Animals showing fasting blood glucose higher than 200mg/dL were considered as diabetic. Myocardial infarction (MI) was produced by Isoproterenol injection (85mg/kg body weight, subcutaneous {s.c.} injection dissolved in normal saline) 24 and 48hrs prior to

scarification (5th weeks). At the end of experimental period, rats were sacrificed for further biochemical investigations as well as histopathological evaluation.

Experimental groups and treatment protocol

Group 1: Normal Control (NC): In Normal Control group, rats were administered distilled water per orally using a feeding cannula for study period of 5 weeks. There were 8 animals in this group.

Group 2: Diabetic ISP Control (D-ISP): The rats were administered distilled water per orally using a feeding cannula for study period of 5 weeks. Streptozotocin (45mg/kg body weight, i.p.) was injected to induce diabetes at 0 week and challenged with Isoproterenol (85mg/kg body weight sc.) 24 and 48hrs prior to scarification. There were 13 animals in this group.

Group 3: Vildagliptin (VIL): Vildagliptin (10mg/kg) was administered orally using a feeding cannula from 1st to 5th week (4 weeks). The streptozotocin (45mg/kg body weight, i.p.) was injected to induce diabetes at 0 week. Subsequently the rats were challenged with Isoproterenol (85 mg/kg body weight s.c.) 24 and 48hrs prior to sacrification. There were 10 animals in this group.

Group 4: *Commiphora mukul* (CM): *Commiphora mukul* (200mg/kg) was administered orally using a feeding cannula from 1st to 5th week (4 weeks). The Streptozotocin (45mg/kg body weight, i.p.) was injected to induce diabetes at 0 week. Subsequently the rats were challenged with Isoproterenol (85 mg/kg body weight sc) 24 and 48hrs prior to sacrification. There were 10 animals in this group.

Evaluation parameters

Assessment of body weight

Each rat was weighed individually twice, first at the beginning of the experiment (initial weight) and second, 24hrs after the administration of the last dose of either drug (final weight). The difference in body weight of each rat was calculated and expressed as percentage change according to the following:

% change in body weight = Final weight-Initial weight X 100/Initial weight

Biochemical parameters

Biochemical parameters were estimated using AU 480 auto-analyzer Backman coulter, Germany. The rat blood samples of all experimental groups were collected from the retro-orbital plexus under light ketamine anesthesia (40mg/kg i.p.) at 0, 1st and 3rd week for estimation of blood glucose and creatinine phosphokinase (CPK-MB). In addition, after the completion of the experimental duration (5th weeks), Serum was used for the determination of the biochemical parameters blood glucose, HbA1c, creatinine phosphokinase (CPK-MB), serum DPP-4, high sensitive C-reactive protein (hs-CRP), pancreatic lipase, serum glutamate pyruvate transaminase (SGPT), creatinine by AU480 autoanalyzer Backman coulter, Germany or ELISA kits in the Pathology (NABL accredited) and Pharmacology laboratory.[13-20]

Histopathological studies

At the end of the experiment (5 weeks), the animals were sacrificed. The heart, pancreas, liver and kidney were immediately fixed in 10% buffered neutral formalin solution. The tissues were carefully embedded in molten paraffin with the help of metallic blocks, covered with flexible plastic moulds and kept under freezing plates to allow the paraffin to solidify. Cross sections (5µm thick) of the fixed tissues were cut. These sections were stained with hematoxyline and eosin and visualized under light microscope to study the microscopic architecture of the tissues.[21] The investigator performing the histological evaluation was blind to biochemical results and to treatment allocation (H and E 40×).

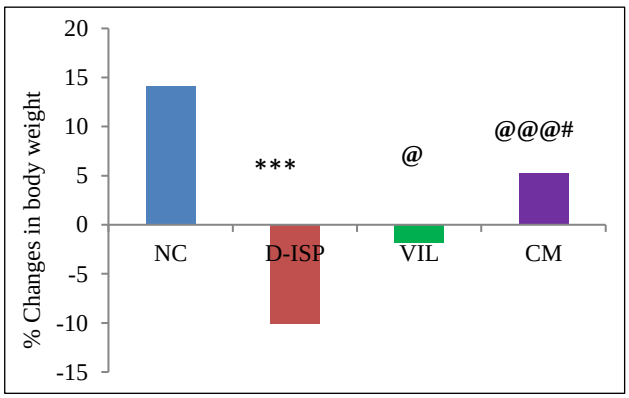
Statistical analysis

All numerical data in text, figures and tables were expressed as the mean+SD. Statistical analysis was performed by One-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Spearman correlation coefficient was used to determine the relationship between CPK-MB and DPP-4. Differences were considered statistically significant at $p<0.05$.

RESULTS

Assessment of body weight changes

Diabetic rats, in general, showed classical symptoms of overt diabetes, with signs such as polydipsia, polyphagia and polyurea and after receiving ISP were beset with, within minutes, extremely rapid respiration.



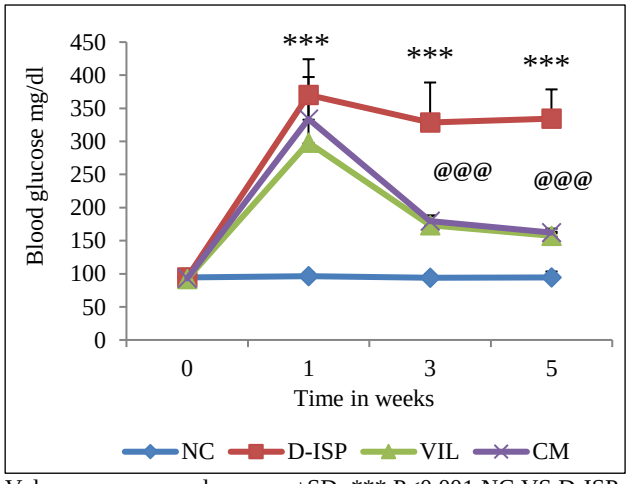
Values are expressed as Mean±SD,***p<0.001NC VS D-ISP; @@@ p<0.001, @ p<0.05 D-ISP VS VIL, CM; #p<0.05 VIL VS CM

Figure-1: Body weight changes among various experimental groups.

It was found that 22 % total mortality in all experimental groups except normal control. Body weight of rats in all the groups was recorded every week and the percentage change in body weight was calculated after 5th week. The percentage change in body weight in NC group rats at the end of 5th week was found to be 14.45, D-ISP:-10.03, VIL:-1.78, CM: 2.37. The D-ISP group rats showed significant decrease in body weight (%) as compared with NC, VIL, CM treated groups. CM treatment showed significant ($p<0.05$) restoration in body weight as compared with VIL group. There were differences in number of rats in various groups of present study because of mortality (Figure 1).

Comparative cardioprotective efficacy of natural and synthetic DPP-4 inhibitors

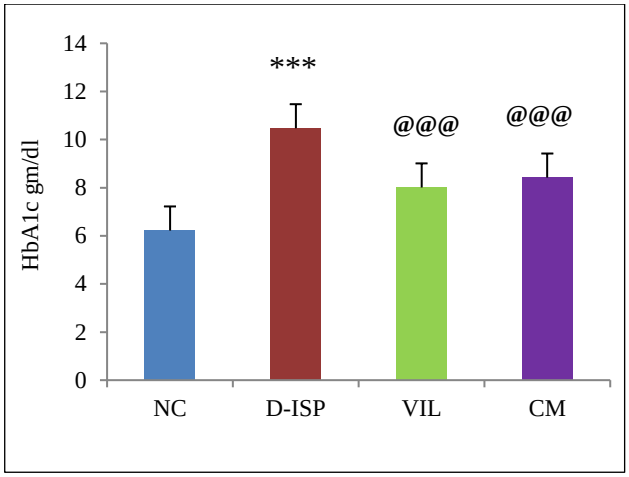
Diabetic parameter: Hyperglycemia induced by Streptozotocin was maintained throughout the study period as evidenced by persistent hyperglycemia throughout the study duration. There was a significant ($p<0.001$) increase in blood glucose and glycosylated hemoglobin levels in D-ISP group rats as compared to NC group. Oral feeding of VIL (10mg/kg) and CM (200mg/kg) significantly restored ($p<0.001$) the elevated blood glucose levels as compared to D-ISP group rats. Similarly, glycosylated hemoglobin was also reduced in treatment group as compared to D-ISP group rats. The antidiabetic efficacy of VIL was found to be comparable to CM therapy (Figure 2,3).



Values are expressed as mean±SD. *** P<0.001 NC VS D-ISP; @@@ p < 0.001 D-ISP VS VIL, CM.

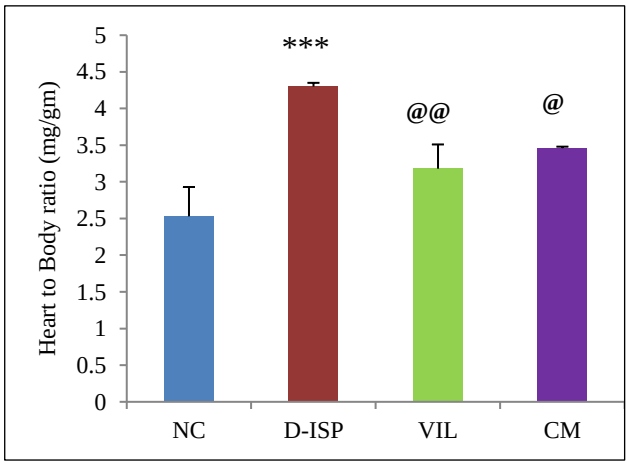
Figure 2: Time course changes (time in week) in blood glucose levels among various experimental groups.

Cardiac parameters: The D-ISP control rats showed significantly increase in ($p<0.001$) heart to body weight ratio as compared to NC rats. The VIL (10mg/kg) ($p<0.01$) and CM (200mg/kg) ($p<0.05$) treatment group rats showed significantly reduced heart to body weight ratio as compared to D-ISP rats. There was no statistical difference between heart to body weight ratio in VIL and CM treatment group rats (Figure 4).



Values are expressed as mean±SD.***p<0.001NC VS D-ISP; @@@ p<0.001 D-ISP VS VIL, CM.

Figure 3: Glycosylated hemoglobin among various experimental groups.



Values are expressed as mean±SD. ***p<0.001 NC VS D-ISP; @@ p<0.01, @p<0.05 D-ISP VS VIL, CM

Figure 4: Heart to body weight ratio among various experimental groups.

There was a significant increase in cardiac markers of injury CPK-MB ($p<0.001$), hs-CRP ($p<0.01$) level in D-ISP rats as compared to NC group at 5th week of study after ISP challenge. VIL (10mg/kg) and CM (200mg/kg) treated group significantly reversed the STZ/ISP induced increase in CPK-MB ($p<0.001$), hs-CRP ($p<0.05$) levels at 5th week. A marked protection against cardiac damage was observed as indicated by decrease in serum CPK-MB isoenzyme, hs-CRP in treated rats as compared to D-ISP group rats. However, the cardioprotective efficacy of the marketed synthetic DPP-4 inhibitor: Vildagliptin was found to be superior to CM ($p<0.05$) (Table 1).

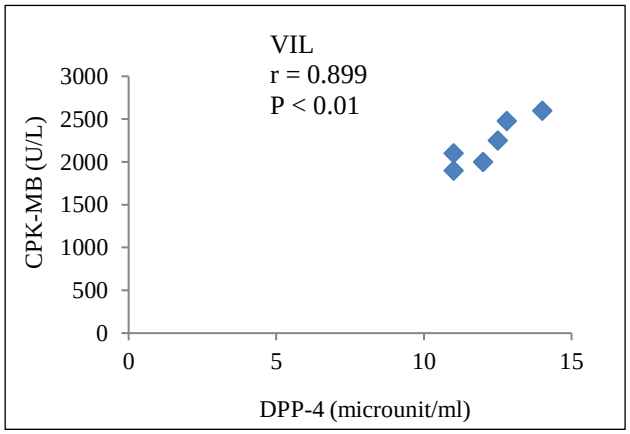
The serum DPP-4 levels ($p<0.001$) increased significantly in D-ISP group rats as compared to NC group rats. VIL (10mg/kg) and CM (200mg/kg) treated rats showed significant reduction in serum DPP-4 levels as compared to D-ISP rats. The VIL (10mg/kg) treated rats showed

significant reduction (p<0.05) in serum DPP-4 levels as compared to CM (200mg/kg) treated rats. Significant cardioprotection as indicated by positive correlation between cardiac marker CPK-MB and serum DPP-4 in VIL (r = 0.899; p<0.01), CM (r = 0.922; p< 0.01) groups was also confirmed by histopathological assessment. (Table 1, Figure 5,6).

Table 1: Cardiac parameters and serum DPP-4 levels among various experimental groups.

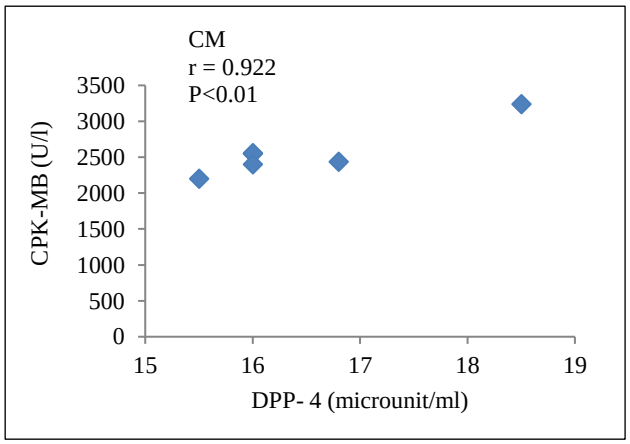
Parameters	Variable	NC	D-ISP	VIL	CM
Cardiac parameters	CPK-MB (U/L)	1565.12±292.07	5424.28±837.73***	2311.25±253.96@@@#	2756.25±458.5@@@
	hs-CRP (mg/dl)	0.86± 0.11	1.9±0.5**	0.91±0.1@@#	1.02±0.1@
DPP-4 Pathway	Serum DPP-4 (microunit/ml)	4.76± 0.43	38.25±4.25***	12.22±1.35@@@#	16.45±1.82@@@

Values are expressed as mean±SD. ***p<0.001 NC VS D-ISP; @@@ p<0.001; @@ p<0.01,@ p<0.05 D-ISP VS VIL,CM; # p<0.05 VIL VS CM



Positive correlation between CPK-MB and serum DPP-4; r = 0.899, P<0.01

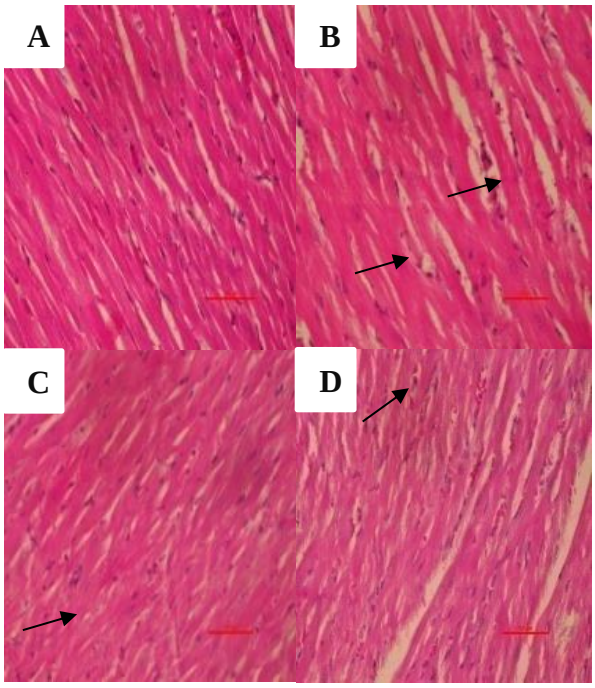
Figure 5: Correlation between CPK-MB and serum DPP-4 of vildagliptin treated group.



Positive correlation between CPK-MB and serum DPP-4; r = 0.922, P<0.01

Figure 6: Correlation between CPK-MB and serum DPP-4 of Commiphora mukul treated group.

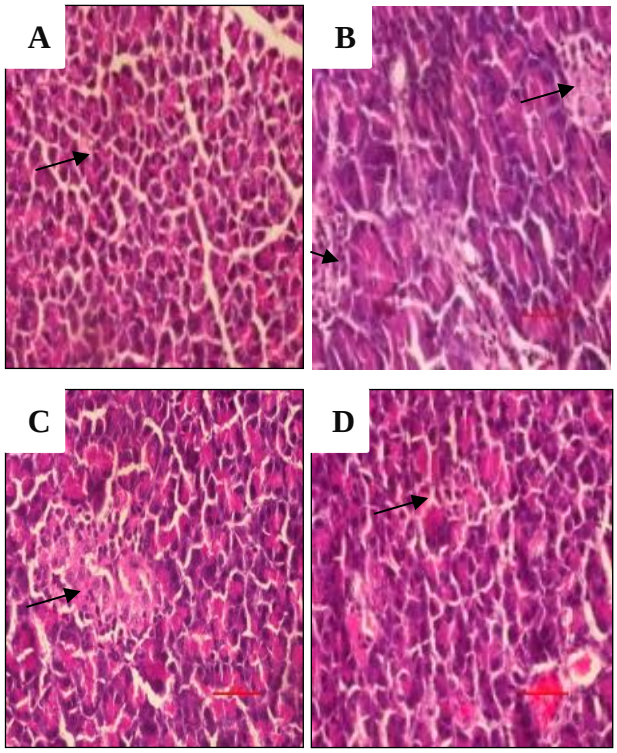
Histopathological section of myocardium: Photomicrograph of NC group rat heart revealed the non-infracted architecture of the myocardium (7A). In contrast,



Arrows indicate separation of myofibers, inflammation. Scale bar = 100μm

Figure 7: Representative photographs demonstrating myocardial tissue sections stained with H and E. (A): Normal control-normal architecture of the myocardium; (B): Diabetic-isoproterenol control-Marked edema, confluent areas of necrosis and separation of myofibers (C): Vildagliptin-less edema was observed; (D): Commiphora mukul occasional focal myofiber loss, less inflammation was observed.

D-ISP group rat heart showed fatty infiltration in myocardial cells, marked edema, confluent areas of myonecrosis, separation of myo-fibers and inflammation as compared to the NC group (7B). In the VIL treatment group rats, occasional focal myofiber loss, less edema was observed (7C). In the CM treatment group rats, occasional focal myofiber loss, less inflammation was observed. However, the degree of edema, inflammation and necrosis were less in treatment groups as compared to the D-ISP. (7D) (Figure 7).



Arrows indicate the beta cells mass. Scale bar = 100μm.

Figure 8: Representative photographs demonstrating pancreatic tissue sections stained with H&E. (A): Normal control-organized pattern and normal architecture of islets of Langerhans and the beta cells mass; (B): Diabetic-isoproterenol control-Damaged islets of langerhans, atrophy of beta cells and reduced beta cell mass; (C): Vildagliptin-improved beta cell mass, less fibrosis, less inflammatory infiltration (D): Commiphora mukul-less fibrosis, less inflammatory infiltration as compared to D-ISP group.

Histopathological section of pancreas: Photomicrograph of pancreatic sections of NC rats showed an organized pattern and normal architecture of islets of Langerhans and the beta cells (8A). In contrast, the pancreas of D-ISP group rat showed severe degenerative changes in the pancreatic islets, damaged islets of Langerhans, reduced beta cell mass and the atrophy of beta cells with the loss of few nucleus and cytoplasm and inflammatory infiltration (8B). Treatment group rats pancreas showed improved beta cell mass, less fibrosis and less inflammatory infiltration as compared to D-ISP group (8C, 8D) (Figure 8).

Safety of natural DPP-4 inhibitors: Commiphora mukul therapy

As seen from the Table 2, it was found that the in D-ISP group a significant elevation in the levels of pancreatic lipase (U/L) ($p<0.001$), SGPT (U/L) ($p<0.001$) and Creatinine (mg/dl) ($p<0.001$) was observed at 5th week compared to NC group. The treatment groups did not adversely affect the pancreatic, liver and kidney function in myocardial infarction co-existing with diabetes rats, as evidenced by pancreatic, hepatic and renal biochemical markers of injury as well as histopathological studies.

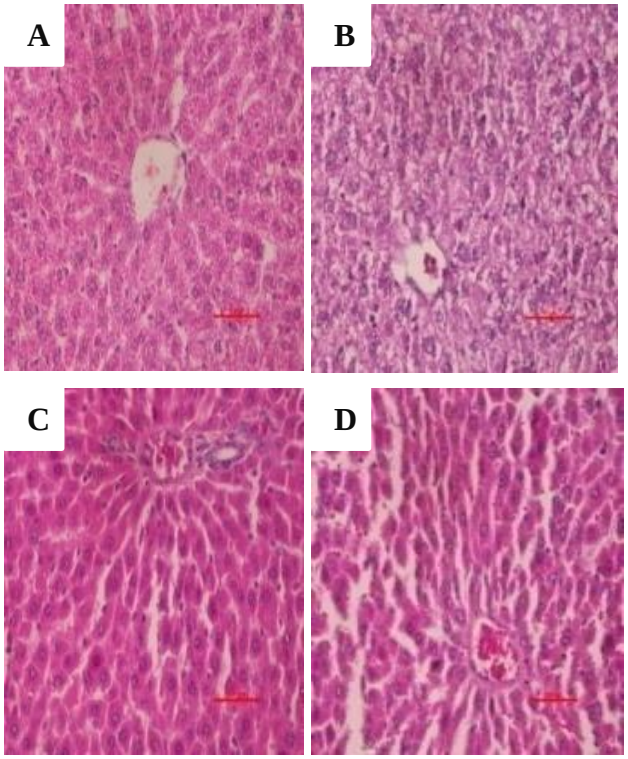


Figure-9: Representative photographs demonstrating histopathological finding of liver tissue sections stained with H&E. (A): Normal control-normal architecture of central vein and hepatocytes; (B): Diabetic-isoproterenol control-scattered necrotic cells, congestion in the central vein; (C): Vildagliptin-less granular degeneration, inflammation; (D): Commiphora mukul-very less granular degeneration and inflammation; scale bar = 100μm.

Histopathological section of liver: Photomicrograph of the liver of the NC group (9A) rats, showed normal architecture of central vein and hepatocytes. In contrast, the liver cells of the D-ISP group (9B) showed degeneration, scattered necrotic cells, congestion in the central vein as compared to NC group. However, VIL treatment (9C) decreased the granular degeneration as compared to D-ISP rats. Peri-portal inflammation, hepatocyte degeneration was less compared to D-ISP Control group. In CM treated group (9D) mild granular degeneration, inflammatory infiltration, hepatocytes degeneration which was less compared to standard drug group was observed. Also, no congestion in central vein was observed (Figure 9).

Histopathological section of kidney: Photomicrograph of NC group kidney (10A) showed normal structure of the kidney, absence of congestion of glomerular blood vessels, tubular necrosis and inflammation. In contrast, histological assessment of the D-ISP group (10B) demonstrated marked congestion of glomerular blood vessels, tubular necrosis and inflammation. In VIL treated group rats kidney showed

Mild tubular necrosis, inflammation (10C). In CM group rats, renal tissue section showed less inflammation and edema. (10D) (Figure 10).

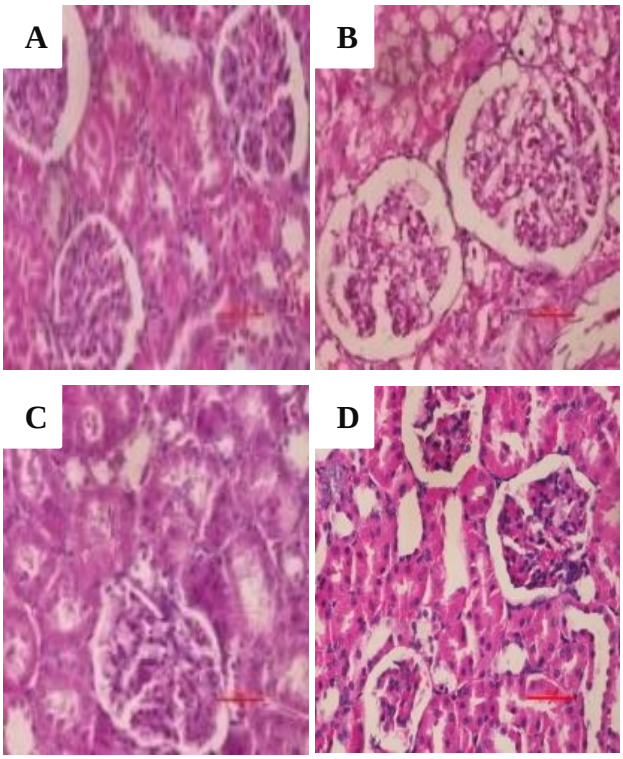


Figure 10: Representative photographs demonstrating histopathological finding of kidney tissue sections stained with H&E. (A): Normal control-normal structure of the kidney; (B): Diabetic-isoproterenol control-marked congestion of glomerular blood vessels, tubular necrosis, inflammation; (C): Vildagliptin-mild tubular necrosis, inflammation; (D): Commiphora mukul-less inflammation and edema; scale bar = 100μm.

Table 2: Safety parameters among various experimental groups.

Vari-ables/ groups	Pancreatic marker lipase (U/L)	Liver marker SGPT (U/L)	Renal marker creatinine (mg/dl)
NC	30.36±1.15	61.25±8.65	0.32±0.07
D-ISP	42.46±4.11***	84.54±5.57***	0.61±0.05***
VIL	38.53±3.62	74.36±8.68@@	0.48±0.07@
CM	32.63±2.51@@@#	66.13±2.56@@#	0.36±0.04@@#

Values are expressed as mean±SD. ***p<0.001 NC VS D-ISP @@@ p<0.001, @ @ p<0.01, @p<0.05 D-ISP VS VIL, CM; #p<0.05 VIL VS CM

DISCUSSION

DPP-4 inhibitors, are a relatively new class of oral hypoglycemic agents, used for treatment of type 2 diabetes mellitus. DPP-4 inhibitors increase the incretin levels (GLP-1 {Glucose dependent insulinotropic polypeptide} and GIP {glucagon-like peptide-1}), which in turn stimulate the insulin secretion and there by lower blood glucose levels. DPP-4 inhibitors work by enhancing the sensitivity of β cells to glucose, and have also shown to improve markers of β cell function.[22] The multiple effects, along with the control of plasma glucose concentrations of DPP-4 inhibitors, represent important mechanisms of action of these drugs. Besides being established as standards antidiabetic effects various preclinical data and mechanistic studies have reported the cardioprotective effects of DPP-4 inhibitors, via GLP and GLP-1 dependent and independent mechanisms. Since a large pool of diabetic patients has cardiovascular co-morbidites, DPP-4 inhibitors may therefore represent promising oral hypoglycemic agents beneficial in this subset of diabetic patients with cardiovascular diseases.

The DPP-4 inhibitors have become widely accepted in clinical practice because of their favorable features including a low risk of hypoglycemia, once daily dosing. However, in spite of their beneficial therapeutic effects, they have few limitations: high cost of therapy and unacceptable adverse effects such as pancreatitis, angioedema, infective disorders, pancreatic cancer, and thyroid cancer.[23-26] In this scenario, it would be beneficial to explore novel DPP-4 inhibitors that share the advantages of DPP-4 inhibition and at the same time are cost effective and safe.

Commiphora mukul, a medicinal plant widely used in Ayurveda has been reported to possess antidiabetic and cardioprotective properties. Previous study reported from the laboratory that *Commiphora mukul*, significantly inhibits DPP-4 enzyme.[27] In the present study, for the first time the cardioprotective efficacy and safety of this natural DPP-4 inhibitor (*Commiphora mukul*) was evaluated in the setting of diabetes using an experimental model of myocardial infarction co-existing with diabetes. The results of the present investigation would provide preliminary experimental evidence based on which further studies can be designed to emphasize the therapeutic benefits of DPP-4 inhibition for diabetic patients with cardiac co-morbidities.

The present study results confirmed the hypoglycaemic effects of hydroalcoholic extract of *Commiphora mukul* in diabetic rats challenged with Isoproterenol. A significant decrease in the blood glucose and HbA1c levels was observed in *Commiphora mukul* and Vildagliptin treated groups as compared to D-ISP control group. Observed antidiabetic activity of *Commiphora mukul* are supported by the previous studies reported by Ramesh B et al, Widad M et al and Barve and Bhonsle. [28-30]

Commiphora mukul and Vildagliptin treatment significantly attenuated cardiac hypertrophy as indicated by a reduction in heart to body weight ratio as compared to D-ISP rats. In addition, the treatment groups restored myocardial CPK-MB, hs-CRP levels as compared to D-ISP group. *Commiphora mukul* and Vildagliptin treatment maintained the membrane integrity of the myocardium and

thus prevented the leakage of CPK-MB into the serum. This result concurs with previous study by Barve and Bhonsle (2014), Philippe O et al (2003) that reported the cardioprotective effects of *Commiphora mukul* in STZ induced diabetic cardiomyopathy in rats.[30,31] The histopathological studies confirmed the cardioprotective effects of *Commiphora mukul* and Vildagliptin treatment. In the *Commiphora mukul* treatment group heart rats, occasional focal myofiber loss, inflammation was observed. However, the degree of injury was less as compared to the D-ISP. The previous study by Barve and Bhonsle (2014) was in agreement with histological findings.[30]

The present study also evaluated the safety of standard drugs and test drugs on the vital organs: pancreas, liver and kidney. The markers of pancreatic function (pancreatic lipase), liver function (SGPT), kidney function (Creatinine) were assessed in addition to histopathological evaluation of the degree of injury. Increased pancreatic lipase levels as seen in D-ISP rats showed presence of pancreatic tissue damage. This was attenuated by various treatment protocols, there by restoring the architecture of the pancreas. This is the first report of the effect of test drugs *Commiphora mukul* on the pancreatic function in the experimental model of myocardial infarction in setting of diabetes. *Commiphora mukul* therapy did not adversely affect the hepatic and kidney function as evidenced by liver and renal function biochemical markers as well as histopathological studies. Earlier report by Taru et al supported that *Commiphora mukul* does not adversely affect liver and kidney function.[32]

Evidence from several studies have suggested that DPP-4 inhibitors improve cardiac function in both animal and clinical studies.[33,2] The study by Connelly KA et al reported that without affecting glycemic control, DPP-4 inhibition increased the abundance of stromal cell-derived factor-1 (SDF-1), enhanced capillary density, reduced cardiac myocyte hypertrophy and improved passive compliance in diastole of diabetic rats with myocardial infarction.[34]

In order to delineate if cardioprotective effects of *Commiphora mukul* is attributed to DPP-4 inhibition, serum DPP-4 levels were estimated in the various experimental groups. *Commiphora mukul* and vildagliptin treatment restored the elevated DPP-4 levels observed in the D-ISP rats. Vildagliptin treatment showed superior reduction in serum DPP-4 levels as compared to *Commiphora mukul*.

It was also found that the cardioprotection (as indicated by cardiac marker CPK-MB levels) demonstrated by *Commiphora mukul* and vildagliptin was found to positively correlate with serum DPP-4 levels indicating that modulation the DPP-4 pathway contributes to their cardioprotective efficacy. Thus, the present study demonstrated the DPP-4 inhibition contributes to the myocardial salvaging effects of *Commiphora mukul* in the setting of diabetes.

This study has some limitations. In the present study, the active chemical compounds of *Commiphora mukul* was not isolated and tested in experimental model of myocardial infarction in setting of Diabetes and also long term toxicity study was not assessed.

CONCLUSION

The natural DPP-4 inhibitor *Commiphora mukul* demonstrated significant cardioprotective effects in experimental model of myocardial infarction co-existing with diabetes.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Borde MK, Mohanty IR, Maheshwari U, Suman RK, Deshmukh YA. DPP-4 inhibitory activity and myocardial salvaging effects of Commiphora mukul in experimental diabetes. Int J Basic Clin Pharmacol 2019;8:575-83.