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Dipeptidyl peptidase IV inhibitory activity of *Commiphora mukul* monotherapy and combination therapy (with Metformin) attributes to its cardioprotective effects in experimental diabetes: *In silico*, *in vitro* and *in vivo* analysis

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Abstract

Objectives: The marketed synthetic Dipeptidyl peptidase-IV (DPP-IV) inhibitors are expensive antidiabetic drugs and have been reported to cause unacceptable adverse effects. In this scenario research to develop novel DPP-IV inhibitors from alternative sources is the need of the hour. *Commiphora mukul*, a medicinal plant with antidiabetic and cardioprotective activities may represent a natural DPP-IV inhibitor, the DPP-IV inhibitory activity of which may translate into demonstrable therapeutic benefits in setting of diabetes with cardiovascular co-morbidities.

Materials and methods: The DPP-IV inhibitory, antidiabetic and cardioprotective effects of *Commiphora mukul* monotherapy and combination therapy (with Metformin) was evaluated in the experimental model of myocardial infarction co-existing with diabetes. To determine the active principle of *Commiphora mukul* responsible for DPP-IV inhibitory activity, the crystal structure of DPP-IV was considered as receptor which was docked against Gluggusterone E, Gluggusterone Z, Sitagliptin and Vildagliptin.

Results: *Commiphora mukul* monotherapy as well as in combination therapy (with Metformin) demonstrated significant DPP-IV inhibitory, antidiabetic and cardioprotective effects in the experimental model of myocardial infarction co-existing with diabetes. Cardioprotective effects of *Commiphora mukul* treatment may be attributed to several mechanisms (DPP-IV inhibition, hypolipidemic, reduced atherogenic potential, anti-thrombotic state, anti-inflammatory, antioxidant and anti-apoptotic activities. The *Commiphora mukul* possesses significant DPP-IV inhibitory activity as delineated, using *in silico* docking.

Conclusion: The present study will provide preliminary experimental evidence on the potential therapeutic benefits of using natural DPP-IV inhibitors in the setting of diabetes co-existing with cardiovascular diseases.

Keywords: DPP-IV inhibitor, *Commiphora mukul*, Combination Therapy, Diabetes, Cardioprotective.

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1. Introduction

All the major classes of oral anti-diabetes medications reduce hyperglycemia, although through different mechanisms with different onsets of action. Reduction of hyperglycemia decreases the risk for microvascular complications (Retinopathy, nephropathy and neuropathy) in patients with type II diabetes mellitus. [1] Nonetheless, a large pool of patients with diabetes dies of macrovascular disease such as cardiovascular diseases.

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

Dipeptidyl peptidase-IV (DPP-IV) inhibitors are a novel class of oral hypoglycemic agents, widely used for the treatment of type II diabetes mellitus. Besides established antidiabetic effects, several studies have reported the cardioprotective benefits of DPP-IV inhibitors via Glucagon-like peptide 1 (GLP-1) dependent and independent pathways. [2] Also, they are known to lower blood pressure, weight neutral, improve dyslipidemia, diminish oxidative stress and improve endothelial function in patients with type II diabetes.[3,4] Therefore, it is plausible to explore the therapeutic potential of DPP-IV inhibitors in the setting of diabetes co-existing with cardiovascular diseases.

The marketed synthetic DPP-IV inhibitors are promising new antidiabetic drugs with potential cardiovascular benefits. However, recent studies reported DPP-IV inhibitors to be causing unacceptable adverse effects such as pancreatitis, angioedema, infective disorders, pancreatic and thyroid cancer. [5-8] Moreover, they are relatively expensive as compared to standard drugs like Metformin, which may make the type II diabetes mellitus patients non-complaint to therapy on a long term basis despite their beneficial effects. In this light, the DPP-IV inhibitors obtained from natural source may be an alternative that needs to be explored, considering the rich biodiversity, India is bestowed with.

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 and diabetes. [9] Although the anti-diabetic, cardioprotective activity of *Commiphora mukul* has been reported, [10,11] its ability to alter the DPP-IV pathway in experimental models had not been studied so far. In this scenario, research to identify novel DPP-IV inhibitors that favorably modify various cardiovascular diseases risk factors, work in concert with the body's own defenses, are less expensive and have fewer side effects, are desirable.

With this point in view, this study was designed to screen natural resources to identify medicinal plants with DPP-IV inhibitory activity which could be further developed as natural alternatives to the synthetic DPP-IV inhibitors. Subsequently the cardioprotective efficacy as well as safety of the natural DPP-IV inhibitors was evaluated in the experimental model of myocardial infarction co-existing with diabetes. Moreover, to unravel the cardioprotective mechanisms, the effect of *Commiphora mukul* on various targets (Atherosclerosis, lipid profile, DPP-IV pathway, apoptosis, inflammation, oxidative-antioxidant balance) was studied. In addition, to determine the possible place of these drugs in therapy, they were used in combination with Metformin and compared with standard anti-diabetic drugs. The present study was designed to delineate the active ingredients of *Commiphora mukul* responsible for its DPP-IV inhibitory activity using *in silico* studies. Thus, the potential of *Commiphora mukul* or its active ingredients to be developed further as natural DPP-IV inhibitors has been emphasized in the present study.

2. Materials and methods

2.1 Experimental animals:

Adult male Wistar rats, 10 to 12 weeks old, weighing 150 to 200 gm were used in the study. Wistar rats were procured from Bombay Veterinary College, BVC Campus Road, Parel, Mumbai. Rats were housed in the Animal Facility of Mahatma Gandhi Mission Medical College, Navi Mumbai, India in polyacrylic cages (38×23×10cm) under standard laboratory conditions. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC/2013/11/3) 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. The animals were allowed free excess to standard diet, tap water *ad libitum* and allowed to acclimatize for one week before the experiments.

2.2 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 from Novartis pharmaceuticals UK Ltd. and Metformin was obtained from Sonafi pharmaceuticals as gift sample. The hydro-alcoholic dried standardized extract of *Commiphora mukul* (Gugglu) was procured from Sanat Pharmaceutical, New Delhi. All other chemicals and reagents used were of analytical grade.

2.3 Standardized hydro-alcoholic dried extract of *Commiphora mukul*:

The pH of 1% w/v aqueous solution of *Commiphora mukul* was found to be 2.92. Loss on drying value at 105°C by infrared balance for *Commiphora mukul* extract was 5.5% w/w. Total ash content for *Commiphora mukul* extract was 7.3% w/w. *Commiphora mukul* extract contained 30% w/w tannins. Total heavy metals assay results of *Commiphora mukul* extract showed that it contained lead 3.0 ppm, arsenic 1.0 ppm, cadmium 1.0 ppm and mercury 0.5 ppm. Microbiological analysis was also done of plant extract. Results of total plate count for *Commiphora mukul* extract: 10.000 colony forming unit/gram. The mould counts of

extracts less than 100 colony forming unit/gram and other microorganism like *E. coli, salmonella, Pseudomonus aeruginosa, Staphylococcus aureus* and yeast were absent in extract.

2.4 Experimentally induced myocardial infarction in setting of Type II diabetes mellitus:

Male Wistar rats weighing 150–200 gm were used for the study. Rakieten *et al*. reported the diabetogenic activity of the compound Streptozotocin. [12] STZ is specifically cytotoxic to beta-cells of the pancreas. Type II Diabetes was induced in rats by a single STZ injection (45mg/kg body wt, i.p. dissolved in 0.01M citrate buffer, pH 4.5) in overnight fasting rats. Serum glucose estimations were undertaken periodically (day 0, 3, 7) from the tail vein to confirm the production of diabetes mellitus. The animals were allowed to drink 5% glucose solution overnight to overcome drug induced hypoglycemia. Animals showing fasting blood glucose higher than 200 mg/dL were considered as diabetic. Myocardial infarction was produced by Isoproterenol (ISP) (85mg/kg dissolved in saline) injection subcutaneous (s.c.) 24 and 48h prior to scarification (5^{th} week). At the end of experimental period, rats were sacrificed, blood sample were collected for further biochemical investigations as well as histopathological evaluation.

2.4 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.

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 (45 mg/kg body weight, i.p.) was injected to induce diabetes at 0 week and challenged with Isoproterenol (85 mg/kg body weight sc.) 24 and 48 h prior to sacrification.

Monotherapy

Group 3: Metformin (MET):

Metformin (100 mg/kg) was administered orally using a feeding cannula from 1^{st} to 5^{th} week (4 weeks). The Streptozotocin (45 mg/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 48 h prior to sacrification.

Group 4: Vildagliptin (VIL):

Vildagliptin (10 mg/kg) was administered orally using a feeding cannula from 1^{st} to 5^{th} week (4 weeks). The Streptozotocin (45 mg/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 48 h prior to sacrification.

Group 5: *Commiphora mukul*(CM):

Commiphora mukul(200 mg/kg) was administered orally using a feeding cannula from 1^{st} to 5^{th} week (4 weeks). The Streptozotocin (45 mg/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 48 h prior to sacrification.

Combination therapy

Group 6:Metformin+Vildagliptin (MET+VIL):

Metformin (100 mg/kg)+Vildagliptin (10 mg /kg) were administered orally using a feeding cannula from $1^{st}$to 5^{th} week (4 weeks). The Streptozotocin (45 mg/kg body weight, i.p.) was injected to induce diabetes at 0 week. Subsequently the rats were challenged with Isoproterenol (85mg/kg body weight sc) 24 and 48 h prior to scarification.

Group 7:Metformin+*Commiphora mukul*(MET+CM):

Metformin (100 mg/kg)+ *Commiphora mukul*(200mg/kg) were administered orally using a feeding cannula from1^{st} to 5^{th} week (4 weeks). The Streptozotocin (45 mg/kg body weight, i.p.) was injected i.p. to induce diabetes at 0 week. Subsequently the rats were challenged with Isoproterenol (85mg/kg body weight sc) 24 and 48 h prior to sacrification.

2.5 Evaluation parameters:

2.5.1 *In vitro* DPP-IV Inhibitory assay:

DPP-IV assay was performed by using DPP-IV assay kit procured from Sigma Aldrich. In this assay, DPP-IV activity was determined by the cleavage rate of 7-amino-4-methylcoumarin (AMC) from the synthetic substrate H-glycyl-prolyl-AMC. One unit of DPP-IV is the amount of enzyme that hydrolyzes the DPP-IV substrate to yield 1.0U mole of AMC per minute at 37°C. The standard curve of free AMC was generated using 0–50mM AMC (Sigma). DPP-IV activity was expressed as the amount of cleaved AMC per minute per ml (nmol/min/ml). The one percent (w/v) extract of *Commiphora mukul* plant in distilled water was used for assay. While Sitagliptin and Vildagliptin were used as a reference drugs and control was prepared without inhibitors/plant extracts. Experiments were done in triplicates. A decrease in DPP-IV activity is measure for inhibition. The percent inhibition was calculated using following formula:

$$\% \text{ inhibition} = \frac{\text{Absorbance of control - Absorbance of inhibitor}}{\text{Absorbance of control}} \times 100$$

2.5.2 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 (40 mg/kg i.p.) at 0, 1^{st} and 3^{rd} week for estimation of blood

glucose and creatinine phosphokinase (CPK-MB). In addition, after the completion of the experimental duration (5^{th} weeks), Serum was used for the determination of the biochemical parameters blood glucose, HbA1c,creatinine phosphokinase (CPK-MB), *B-type* natriuretic peptide (BNP),serum DPP-IV, high sensitive C-reactive protein (hs-CRP), lipid profile, serum thiobarbituric acid reactive substances (TBARS), Fibrinogen level, 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.

2.5.3 Histopathological evaluation:

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 (5 µm 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. The investigator performing the histological evaluation was blind to biochemical results and to treatment allocation. (H&E 40×)

2.5.4 Estimation of serum DPP-IV activity:

DPP-IV assay was performed by using DPP-IV assay kit procured from Sigma Aldrich Chemical Co., St Louis, USA. DPP-IV assay was performed according to the method of Almasri *et al* with slight modifications. [13] In brief, 10 µl of serum of samples from different groups, 10 µl of DPP-IV Assay buffer and 10 µl of the DPP-IV inhibitor were added to each of the sample wells. The mixture was incubated for 10 minutes at 37°C, followed by addition of the reaction mixture. After gently mixing, the plate was incubated at 37°C. The initial and final fluorescence intensity was taken with the help of fluorescence HPLC Detector (FLD-3000 Fluorescence Detector) and DPP-IV activity was calculated.

2.5.5 Determination of Myocardial Apoptosis:

The heart was immediately fixed in 10% buffered neutral formalin solution after scarification. Myocardial apoptosis was quantitatively analyzed by detection of DNA fragmentation using a commercially available TUNEL assay kit (Promega Co USA), The Dead End™ Colorimetric TUNEL System end-labels the fragmented DNA of apoptotic cells. Biotinylated nucleotide was incorporated at the 3′-OH DNA ends using the Terminal Deoxynucleotidyl Transferase, Recombinant, (rTdT) enzyme. Horseradish peroxidase-labeled streptavidin (Streptavidin HRP) is then bound to these biotinylated nucleotides, which was detected using the peroxidase substrate, hydrogen peroxide, and the stable chromogen, diaminobenzidine (DAB). Using this procedure, apoptotic nuclei was stained dark brown. The slides were then visualized under light microscope to study the apoptotic cells i.e. TUNEL positive cells.

2.5.6 *In silico* DPP-IV inhibitory activity of active ingredients of *Commiphora mukul:*

The crystal structure of human DPP-IV (PDB Id: 2QT9) was downloaded from Protein Databank which has a resolution of 2.1Å, which was considered as a receptor for docking studies.[14] The ligands selected included active ingredients of *Commiphora mukul* (Gluggusterone E, Gluggusterone Z), Sitagliptin and Vildagliptin. All of them were downloaded from Pubchem Database.[15] All these structures were retrieved in SDF file format which was further converted into 3D format using Frog v2.14 FRee On line druG conformation generation.[16] The active site of DPP-IV was retrieved through literature search and also predicted based on CASTp online server to identify pockets.[17] Now the receptor and the 3D generated ligands were considered for docking using Hex software 8.0.0.[18] It is an interactive molecular graphics program for calculating and displaying feasible docking modes which uses spherical polar Fourier (SPF) correlations to accelerate the calculations. The 2QT9 was loaded along with their inhibitor 4-aryl cyclohexylalanine in complex state. Top ten docked poses were downloaded and considered for further analysis using Swiss pdb viewer and CHIMERA software. [19]

2.6 Statistical Analysis:

The Data were analyzed by student t test One –way analysis of variance (ANOVA) and values less than $p < 0.05$ were considered as statistically significant. Spearman correlation coefficient was used to determine the relationship between different variablesDifferences were considered statistically significant at $p<0.05$.

3. Results

3.1 In vitro DPP-IV inhibitory activity:

The 1% hydroalcoholic extracts of *Commiphora mukul* was screened for dipeptidyl peptidase-IV inhibitory activity by in vitro assay. The hydroalcoholic extracts of *Commiphora mukul* showed 92.97±8.45% DPP-IV inhibitory activity. The one percent hydroalcoholic extract of medicinal plant extracts *Commiphora mukul* was compared with the synthetic DPP-IV inhibitors Vildagliptin & Sitagliptin by in vitro DPP-IV assay. The dipeptidyl peptidase-IV inhibitory activity of synthetic drugs Vildagliptin and Sitagliptin were found to be 90.42±7.84% and 84.67±8.21% respectively. There was no statistically significant difference in DPP-IV inhibitory activity between the groups. *Commiphora mukul* showed DPP-IV inhibitory activity, reflecting the potential benefits of developing indigenous DPP-IV inhibitors. (Fig. 2)

3.2 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 monotherapy (MET, VIL and CM) and combination therapy (MET+VIL and MET+CM) 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 MET+VIL ($p<0.05$) was found to be superior as comparable to MET+CM and monotherapy. (Figure 3 ,4)

3.3 Cardiac parameter:

The D-ISP control rats showed significantly increase in ($p<0.001$) heart to body weight ratio, cardiac markers of injury CPK-MB ($p<0.001$), hs-CRP ($p<0.01$) level, BNP and Atherogenic index as compared to NC rats. The monotherapy MET, VIL & CM and combination therapy MET+VIL, MET+CM 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 & CM treatment group rats. (Figure 4) MET+VIL (100+10 mg/kg) combination significantly ($p<0.05$) reduced heart to body weight ratio as compared to monotherpy and MET +CM. (Figure 4) The treatment group significantly reversed the STZ/ISP induced increase in CPK-MB ($p<0.001$), hs-CRP ($p<0.001$,$p<0.05$),BNP ($p<0.05$) levels at 5th week. A marked protection against cardiac damage was observed as indicated by decrease in serum CPK-MB isoenzyme, hs-CRP and BNP level in treated rats as compared to D-ISP group rats. However, the cardioprotective efficacy of the marketed synthetic DPP-IV inhibitor: Vildagliptin combination with Metformin was found to be superior than monotherpy and MET+CM. (Table 1) The significant increase in Atherogenic index in D-ISP rats after ISP challenge suggests increased risk of atherogenicity. Atherogenic index was also significantly reduced in treatment group rats as compared to D-ISP group. Interestingly, the atherogenic index was most favorably restored in MET+CM ($P<0.05$) as compared to MET. (Figure 5) Cardioprotective efficacy of the treatment groups was also confirmed by histopathological assessment.

3.4 Mechanism of action of *Commiphora mukul*

3.4.1 DPP-IV pathway:

The serum DPP-IV activity ($P<0.001$) increased significantly in D-ISP group rats as compared to NC group rats. Oral treatment of respective group rats showed significant reduction in serum DPP-IV levels as compared to D-ISP group rats. The MET+VIL treated rats showed superior reduction in serum DPP-IV levels followed by MET+CM,VIL,CM,MET treated rats respectively. Significant cardioprotection as indicated by positive correlation between cardiac marker CPK-MB and serum DPP-IV in VIL ($r = 0.899$; $p < 0.01$), CM ($r = 0.922$; $p < 0.01$) groups was also confirmed by histopathological assessment. (Table 1)

3.4.2 Prothrombotic state:

Prothrombotic state indicated by Fibrinogen level significantly increased in D-ISP group as compared to NC. In combination group (MET+VIL, MET+CM) fibrinogen level were significantly reduced as compared to metformin group. (Figure 6)

3.4.3 Inflammation:

The inflammatory (hs-CRP) marker ($p<0.01$) increased significantly in D-ISP group rats as compared to NC group rats. The monotherapy and combination treatment group rats showed significant reduction in inflammatory (hs-CRP) marker as compared to D-ISP group rats. MET+VIL treated rats showed superior reduction in inflammatory (hs-CRP) marker as compared to other treated group rats. (Table 1)

3.4.4 Oxidative stress (lipid peroxidation marker: TBARS)

The oxidative stress induced lipid peroxidation marker TBARS ($p<0.01$) increased significantly in D-ISP group rats as compared to NC group rats. Oral treatment of respective monotherapy and combination groups rats showed significant reduction in serum TBARS level as compared to D-ISP group rats at 5th week. MET+ CM combination therapy attenuated the deleterious effects of STZ/ ISP by significantly reducing lipid peroxidation. (Figure 7)

3.4.5 Dislipidemia:

The total cholesterol, triglyceride, low-density lipoprotein were significantly ($p<0.001$) increased in D-ISP group as compared with NC group at the end of 5th weeks. High density lipoprotein was significantly decreased in D-ISP group rats as compared with NC group. In treatment groups group rats total cholesterol, triglyceride, and low-density lipoprotein were significantly lower ($p<0.001$) as compared to D-ISP group rats. However high density lipoprotein was significantly increased in these treatment groups. MET+ CM treated rats favorably ($p<0.05$) modulated lipid parameters.

3.4.6 Myocardial Apoptotic changes:

The terminal deoxynucleotidyl transferase-mediated DNA nick-end labeling staining was used to identify the existence of myocardial apoptosis in the setting of myocardial infarction co-existing with diabetes. The myocardium tissue sections were stained with nick end labeling (TUNEL) for DNA breaks in different experimental groups. TUNEL positive cells were expressed as percentage of total myocytes.

Present study showed that TUNEL positive nuclei were significantly increased in the D-ISP (34.5± 3.83%) group as compared to NC group (1.83± 0.2%). (Plate 8A, 8B) Diabetes rats challenged with ISP demonstrated the presence of enhanced apoptotic cell death. The TUNEL positivity was significantly reduced in the treatment groups as compared with D-ISP. Additionally, a significant decrease in the number of apoptotic cells in combination group was observed as compared with standard drugs MET group. The apoptotic cells in the treatment groups were: MET (16.8 ±1.68%), VIL (18.8 ±1.7%), CM (12.4 ±1.37%), MET+VIL (8.4 ±0.96%) and MET+CM (7.6 ±0.84%). (Figure 8, Plate 8A-G).

3.5 Histopathological section of myocardium:

Photomicrograph of NC group rat heart revealed the non-infracted architecture of the myocardium (Plate 9A). In contrast, D-ISP group rat heart showed fatty infiltration in myocardial cells, hemorrhage, marked edema, confluent areas of myonecrosis, separation of myo-fibers, congested blood vessels and inflammation as compared to the NC group. (Plate 9B) In the MET and VIL treatment group rats, less inflammation, necrosis and edema was observed. (Plate 9C-D) In the CM treatment group rats, occasional focal myofiber loss, inflammation, necrosis and edema was observed (Plate 9E). In combination therapy, Mild edema and very less separation of myofibers was observed in MET+VIL, MET+ CM. However, the degree of edema, inflammation and necrosis was less as compared to the monotherapy. (Figure 9, Plate 9F & 9G)

3.6 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 (Plate 10A). 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 (Plate 10B). Treatment group rats pancreas showed improved beta cell mass, less fibrosis, less inflammatory infiltration and hemorrhage as compared to D-ISP group (Plate 9C,D,E,F and G) (Figure 10, Table 2)

3.7 Safety of natural DPP-IV inhibitors: *Commiphora mukul* therapy:

As seen from the table 2, it was found that in the 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.

3.7 Histopathological section of Liver:

Photomicrograph of the liver of the NC group (Plate 11A) rats, showed normal architecture of central vein, peripheral vein and hepatocytes. In contrast, the liver cells of the D-ISP group (Plate 11B) showed degeneration, scattered necrotic cells, congestion in the central vein as compared to NC group. However MET treatment (Plate 11C) decreased the granular degeneration as compared to D-ISP rats. Peri-portal inflammation, hepatocyte degeneration was less compared to D-ISP Control group. Similar results were observed with VIL, MET+VIL (Plate 11D, E) treatment. In CM treated group (Plate 11F) mild granular degeneration, inflammatory infiltration, edema, necrosis, hepatocytes degeneration which was less compared to standard drug group was observed. Also no congestion in central vein was observed. The MET+CM (Plate 11F, 11G) group rats liver showed mild inflammatory infiltration, edema and normal structure of central vein, peripheral vein with no congestion. (Figure 11)

3.8 Histopathological section of Kidney:

Photomicrograph of NC group kidney (Plate 12A) 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 (Plate 12B) demonstrated marked congestion of glomerular blood vessels, tubular necrosis, inflammation and cloudy degeneration. The MET treated rats kidney showed congestion of glomerular blood vessels, less hemorrhage, tubular necrosis, inflammation and focal area as compared to D-ISP group rats. (Plate 12C). In VIL, CM treated group rats kidney showed congestion of glomerular blood vessels, inflammation and focal area. Similar results were observed with MET+VIL group rats. (Plate 12D-F). MET+CM treated group (Plate 12G) renal tissue section showed no congestion of glomerular blood vessels, mild inflammation. (Figure 12)

***3.9 In silico* study on DPP-IV inhibitory activity of active ingredients of *Commiphora mukul*:**

In this study, the crystal structure of DPP-IV was considered as receptor which was docked against compounds. DPP-IV is active in homo dimer form. The active site is a deep cleft in DPP-IV which can be accessed via the opening of the propeller domain or through side opening formed at the interface of the β-propeller and hydrolase domains. Furthermore, the β propeller is a funnel shaped tunnel which extends to the active site. All these chemical compounds were docked in the active site pocket. *Commiphora mukul* (Gluggusterone E, Gluggusterone Z) possessed significant DPP-IV inhibitory activities. Gluggusterone E, Gluggusterone Z prefers the active site pocket. (Figure.13 E &13F) Of these, in particular, Gluggusterone E, Gluggusterone Z, very close to amino acid residues Glu205

and Glu206. Here we observed that, Gluggusterone E and Gluggusterone Z, preferred to bind within the active pocket, whereas Vildagliptin prefer to interact with interface region, and Sitagliptin binds to α/β hydrolase domains. The synthetic DPP-IV inhibitors: Sitagliptin binds to amino acids: Glu452 and Vildagliptin to Asp739. The active ingredients of *Commiphora mukul,* Gluggusterone E, Gluggusterone Z bind to same amino acid Asn710. Based on the binding energy results, it was found all the ligands i.e. active ingredients of medicinal plants had superior DPP-IV binding affinity as compared to Sitagliptin and comparable to Vildagliptin. (Table 3)

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

S. N	Parameters	Variable	NC	D-ISP	MET	VIL	CM	ME T+VIL	MET+CM
1	Cardiac parameters	CPK-MB (U/L)	1565.12± 292.07	5424.28± 837.73***	2608.57± 345.27 @@@	2311.25± 253.96@@@#	2756.25± 458.5@@@	1885± 524.86 @@@%%	2037.5± 319.31@@@%%
2		hs-CRP (mg/dl)	0.86± 0.11	1.9 ± 0.5**	0.98± 0.23@	0.91±0.1@@#	1.02± 0.1 @	0.87± 0.24@@	0.88± 0.12@@
3		BNP	0.6+ 0.07	2.76± 0.25***	1.7±0.15@	-	-	0.9± 0.08@@@%	1.1±0.1@@
4	DPP-IV Pathway	Serum DPP-IV (microunit/ml)	4.76± 0.43	38.25± 4.25***	24.76 ± 2.47@@	12.22± 1.35@@@#	16.45± 1.82@@@	7.5± 0.83@@@%!	9.24± 1.02@@@

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

Table 2: Safety parameters among various experimental groups

S. No	Variables/ Groups	Pancreatic Marker Lipase(U/L)	Liver Marker SGPT(U/L)	Renal Marker Creatinine (mg/dl)
1	NC	30.36±1.15	61.25±8.65	0.32±0.07
2	D-ISP	42.46±4.11***	84.54±5.57***	0.61±0.05***
3	MET	35.63±1.26@@	73.17±5.19@@	0.45±0.08@@
4	VIL	38.53±3.62	74.36±8.68@@	0.48±0.07@
5	CM	32.63±2.51@@@#	66.13±2.56@@#	0.36±0.04@@#
7	MET+VIL	36±2.3@@@	78.76±4.58@%	0.51±0.09@%
8	MET+CM	33.8±1.2@@@	68.63±5.37@@%!	0.4±0.05@@

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

Table 3: Details of Standard & Natural DPP-IV inhibitors with their IC50 value, binding energy and their interacting residues against DPP-IV

Sr. No.	Chemical compound	IC50	Binding energy	Amino acids
Commiphora mukul				
1	Gluggusterone E	15 µM	-239.64	Asn710
2	Gluggusterone Z	17 µM	-239.64	Asn710
Standard DPP-IV inhibitors				
3	Sitagliptin	18nm	-139.91	Glu452
4	Vildagliptin	3nm	-237.57	Asp739

Figure 1: Protocol summary

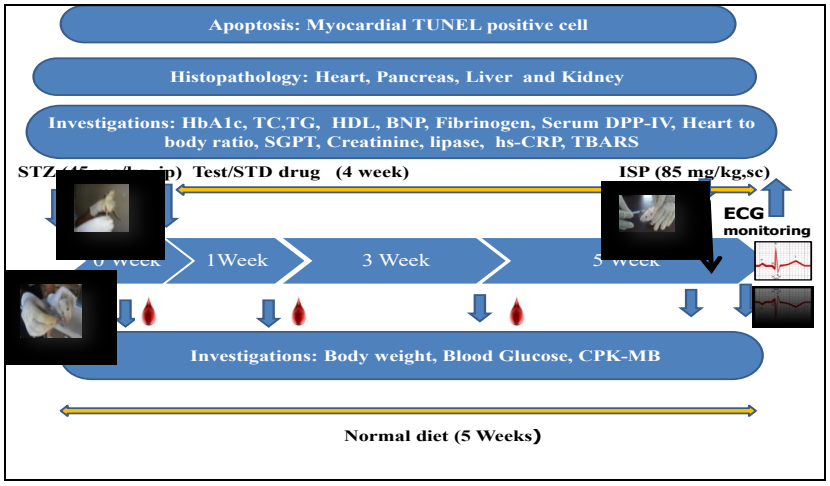
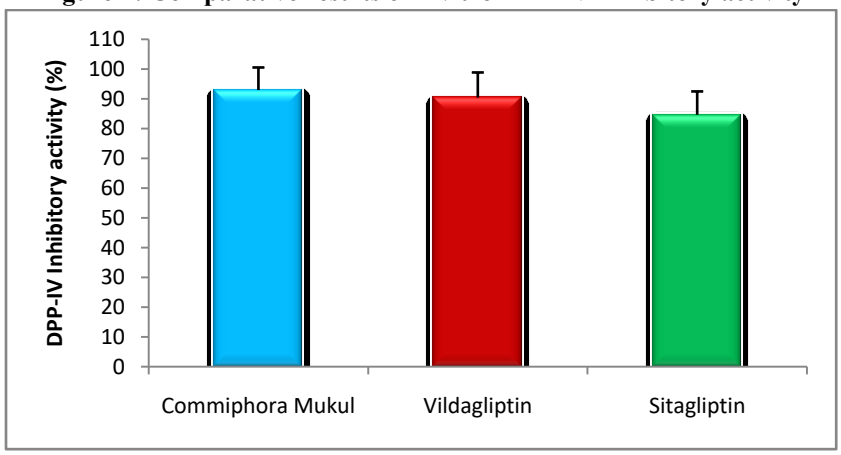


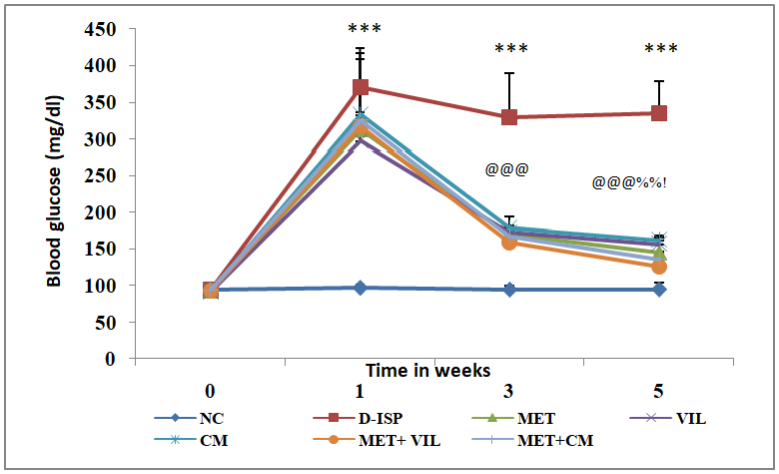
Figure 1: Protocol summary; STZ: Streptozotocin; ISP: Isoproterenol; CPK-MB: Creatinine phosphokinase-MB; HbA1c:Glycosylated haemoglobin A1c; TC: Total cholesterol; TG: Triglyceride; HDL-C:Hight density lipoprotein cholesterol; BNP:*B-type* natriuretic peptide; SGPT: Serum glutamic-pyruvic transaminase; hs-CRP:High sensitive reactive protein; TBARS: Thiobarbituric acid reactive substances; DPP-IV:Dipeptidyl peptidase-IV; TUNEL: Terminal Deoxyribonucleotidyl Transferase-Mediated dUTP Nick End Labeling

Figure 2: Comparative results of invitro DPP-IV Inhibitory activity



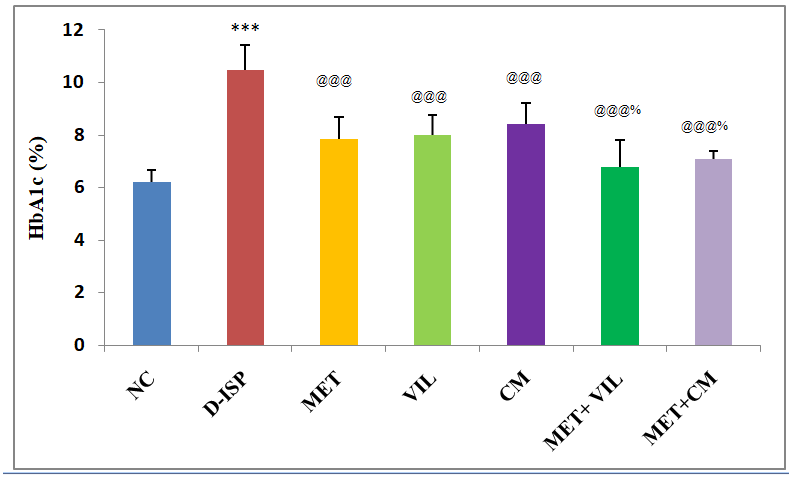
Each vertical bar represents the mean±SD.

Figure 3: Time course changes (time in week) in blood glucose levels among various experimental groups



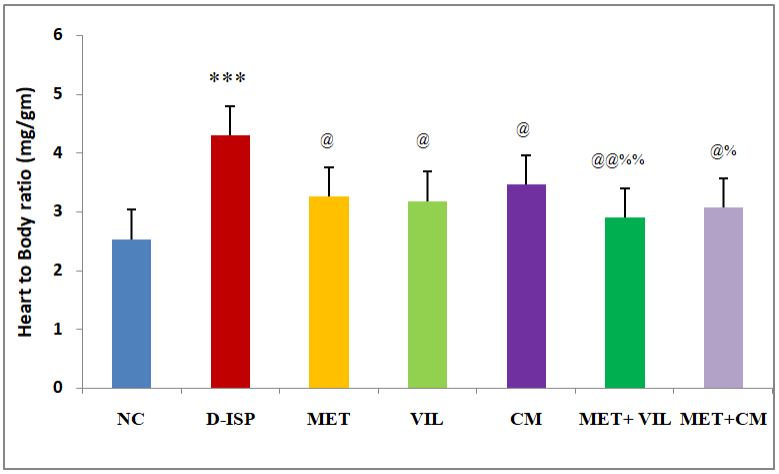
Values are expressed as mean±SD. *** P<0.001 NC VS D-ISP; @@@ p < 0.001 D-ISP VS MET, VIL, CM, MET+VIL, MET+CM; %%p<0.01MET VS MET+VIL, MET+CM; !p<0.05 MET+VIL VS MET+CM.

Figure 4: Glycosylated hemoglobin level among various experimental groups



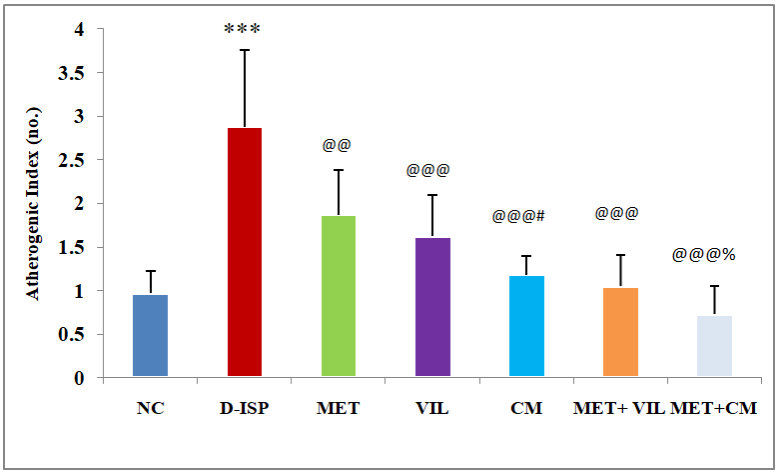
Values are expressed as mean±SD. *** P<0.001 NC VS D-ISP; @@@ p < 0.001 D-ISP VS MET, VIL, CM, MET+VIL, MET+CM; %p<0.01MET VS MET+VIL, MET+CM.

Figure 5: Heart to body weight ratio among various experimental groups



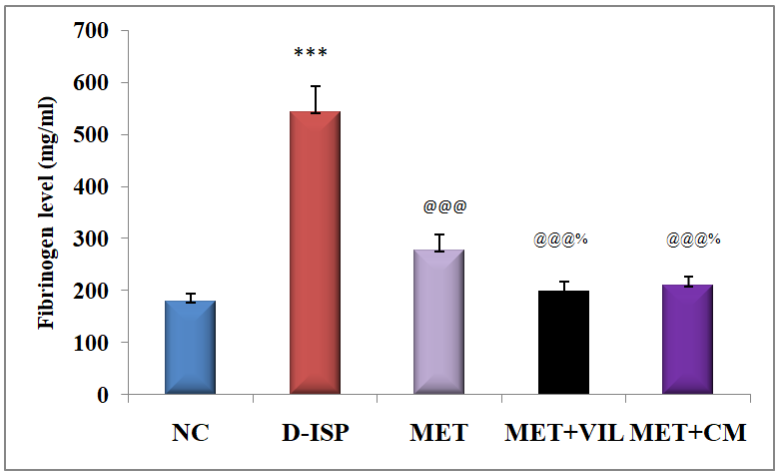
Values are expressed as mean±SD. *** P<0.001 NC VS D-ISP; @@@ p < 0.01@@ p < 0.001@p<0.05 D-ISP VS MET,VIL, CM,MET+VIL, MET+CM; # p< 0.05 VIL VS CM; %%p,0.01,%p<0.05MET VS, MET+CM,MET+VIL

Figure 6: Atherogenic index among various experimental groups



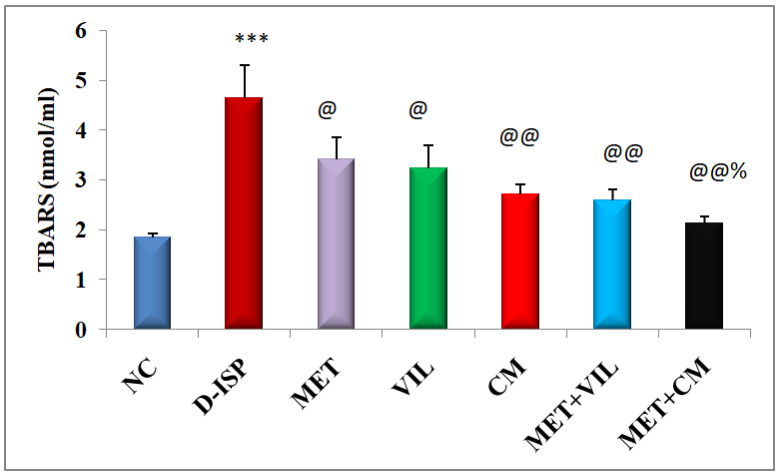
Values are expressed as mean±SD. *** P<0.001 NC VS D-ISP; @@@ p < 0.01@@ p < 0.001@p<0.05 D-ISP VS MET,VIL, CM,MET+VIL,MET+CM; # p< 0.05 VIL VS CM; ,%p<0.05MET VS MET+MET

Figure 7: Fibrinogen levels among various experimental groups



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

Figure 8: TBARS levels among various experimental groups



Values are expressed as mean ±SD. @@p<0.01 D-ISP VS MET, VIL, CM, TA, MET+VIL, MET+CM; % p<0. 05 MET VS MET +VIL, MET +CM.

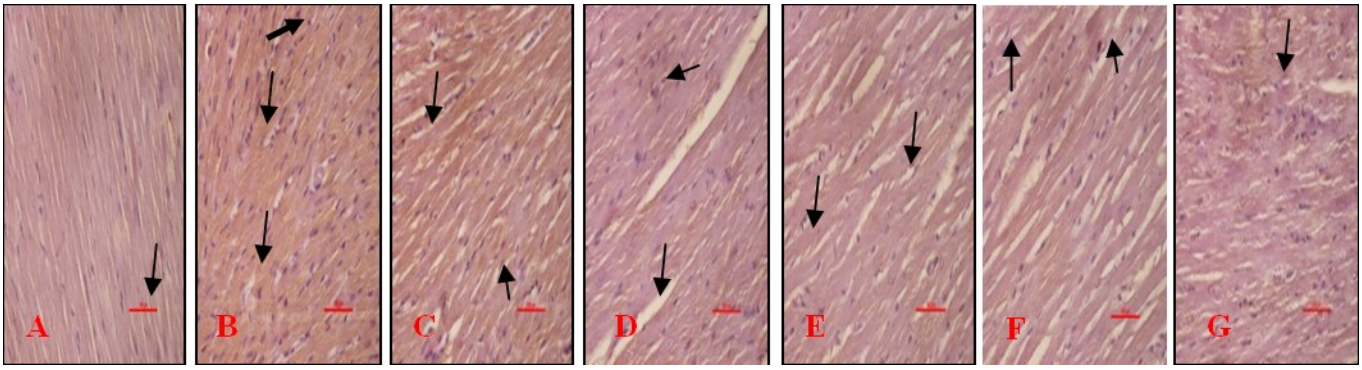


Figure 9: Representative photomicrographs demonstrating myocardial tissue sections stained for apoptosis using TUNEL Assay in the different experimental groups. Scale bar = 100 µm.

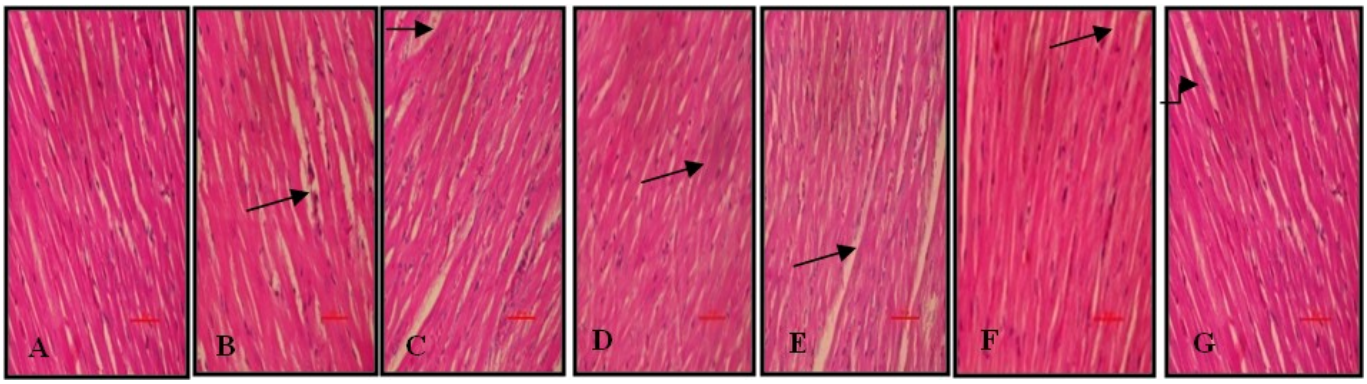


Figure 10: A-G: Representative photographs demonstrating myocardial tissue sections stained with H&E among various experimental groups. (A): Normal control- Normal architecture of the myocardium; (B): Diabetic –Isoproterenol control-Marked edema, confluent areas of necrosis and separation of myofibers, congested blood vessels, inflammation and haemorrhage; (C): Metformin- Less occasional focal myofiber loss, less inflammation and edema; (D): Vildagliptin – Less inflammation, edema; (E): *Commiphora mukul* c –Occasional focal myofiber loss, less inflammation, necrosis and edema; (F): Metformin+Violdagliptin- Mild edema and very less separation of myofibers; (G): Metformin+*Commiphora mukul* – Less inflammation and less separation of myofibers. Arrows indicate separation of myofibers, inflammation. Scale bar = 100 µm.

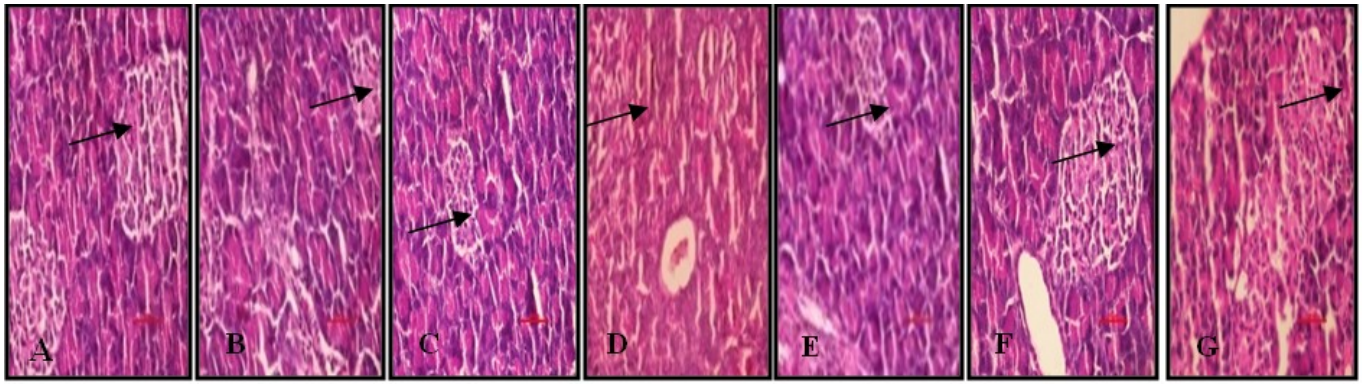


Figure 11: A-G: Representative photographs demonstrating pancreatic tissue sections stained with H&E among various experimental groups. (A): Normal control- Organized pattern and normal architecture of islets of langerhans and the beta cells mass; (B): Diabetic –Isoproterenol control-Damaged islets of langerhans, atrophy of beta cells and reduced beta cell mass; (C):Metformin- Improved beta cell mass, less inflammatory infiltration and edema; (D): Vildagliptin - Improved beta cell mass, less inflammatory infiltration and no hemorrhage; (E): *Commiphora mukul*- Improved beta cell mass, less fibrosis, less inflammatory infiltration as compared to D-ISP group. (F): Metformin+Vildagliptin- Marked improved beta cell mass, less inflammatory infiltration; (G):Metformin+ *Commiphora mukul* - Improved beta cell mass. Arrows indicate the beta cells mass. Scale bar = 100 µm.

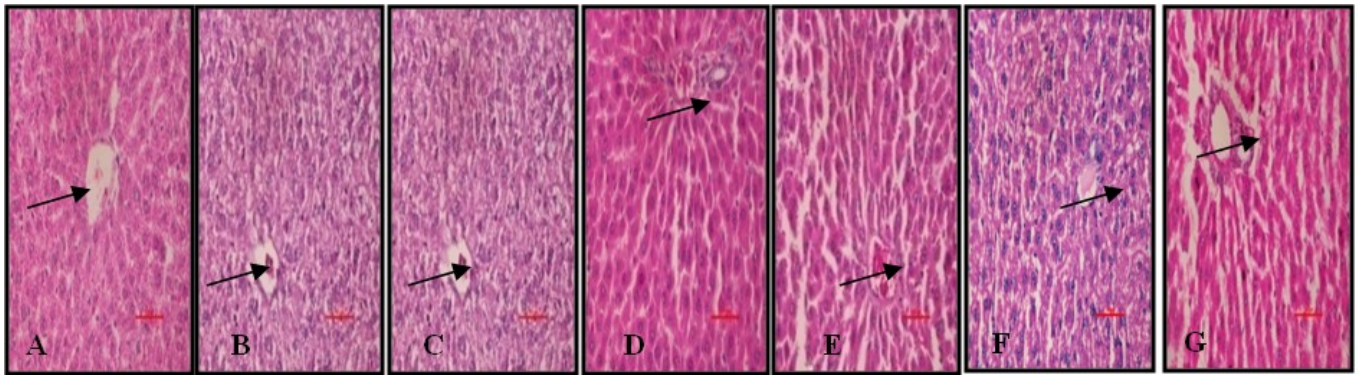


Figure 12:A-G: Representative photographs demonstrating histopathological finding of liver tissue sections stained with H&E among various experimental groups (A): Normal control- Normal architecture of central vein, peripheral vein & hepatocytes; (B): Diabetic– Isoproterenol control-scattered necrotic cells, congestion in the central vein; (C) : Metformin - Less granular degeneration, Peri-portal inflammation & necrosis.; (D) :Vildagliptin- Less granular degeneration, inflammation and necrosis; (E):*Commiphora mukul* – Very Less granular degeneration, inflammation, edema, necrosis; (F): Metformin + Vildagliptin - Less hepatocytes degeneration, peri-portal inflammation, mild edema; (G): Metformin+ *Commiphora mukul* - Mild inflammatory infiltration, normal structure of central vein. Arrows indicate the central vein with radiating cords of hepatocytes; Portal tract. Scale bar = 100 µm.

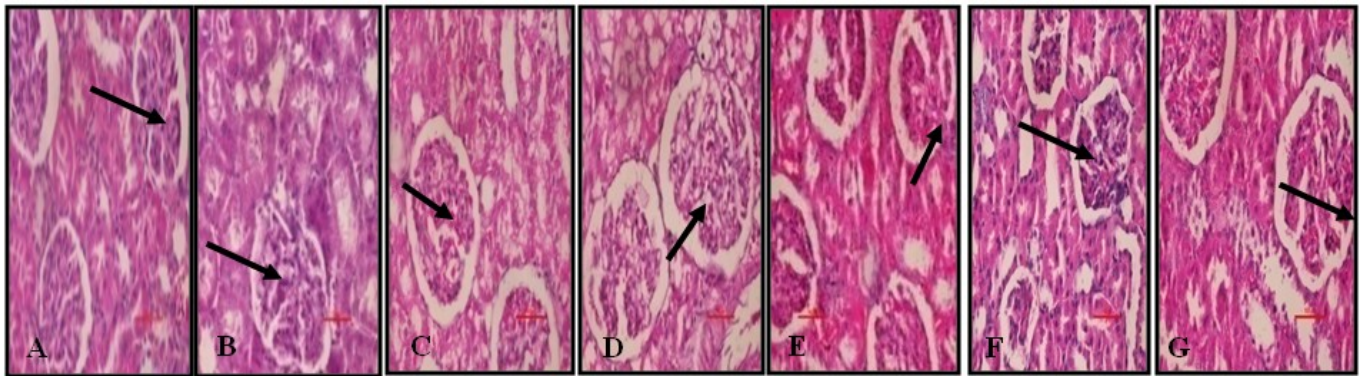


Figure 13: A-G: Representative photographs demonstrating histopathological finding of kidney tissue sections stained with H&E among various experimental groups. (A): Normal control- Normal structure of the kidney; (B): Diabetic–Isoproterenol control- Marked congestion of glomerular blood vessels, tubular necrosis, inflammation and cloudy degeneration; (C): Metformin – Less congestion of glomerular blood vessels, tubular necrosis, inflammation and focal area. (D): Vildagliptin- Mild tubular necrosis, inflammation and edema; (5C):*Commiphora mukul* – less tubular necrosis and inflammation; (F) Metformin + Vildagliptin - Mild congestion of glomerular blood vessels, less tubular necrosis and inflammation. (G): Metformin+ *Commiphora mukul* – Very mild edema and inflammation. Arrows indicate the Glomerulus; Distal convoluted tubules; Proximal convaluated tubule; Scale bar = 100 µm.

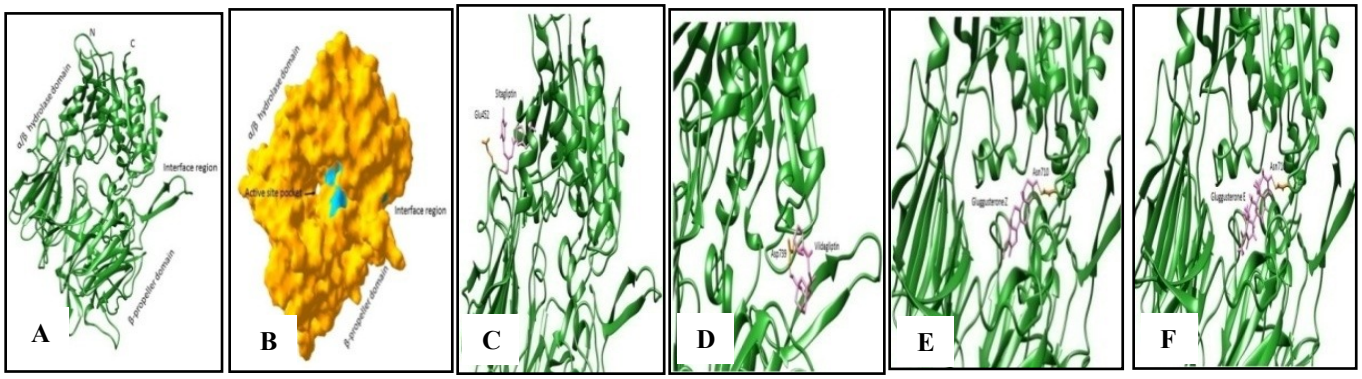


Figure 14: 14A: The crystal structure of DPP-IV, 14B: the Ribbon format showing the α/β hydrolase domain, β propeller region, interface region & the active site pocket in blue colour; 14C & D: Docked poses of DPP-IV against the drugs Vildagliptin & Sitagliptin; 14E & F: The docking of active ingredients of *Commiphora mukul* in the active site pocket of DPP-IV receptor: Gluggusterone Z, Gluggusterone E.

4. Discussion

DPP-IV inhibitors are a relatively new class of oral hypoglycemic agents for treatment of type II diabetes mellitus. Besides being established as standards antidiabetic effects various preclinical data and mechanistic studies have reported the cardioprotective effects of DPP-IV inhibitors, via GLP and GLP-1 dependent and independent mechanisms.[2] Since a large pool of diabetic patients has cardiovascular co-morbidites, DPP-IV inhibitors may therefore represent promising oral hypoglycemic agents beneficial in this subset of diabetic patients with cardiovascular diseases. However, in spite of their beneficial therapeutic effects, they have few limitations, high cost of therapy and unacceptable adverse effects. With this point of view the study was designed to explore novel DPP-IV inhibitors, from natural source that share the advantages of DPP-IV inhibition and at the same time are cost effective and safe.

Commiphora mukul possesses a vast ethno medical history and represents a photochemical reservoir of heuristic medical value. Although the anti-diabetic activity of *Commiphora mukul* has been reported [20], its ability to alter the DPP-IV pathway had not been studied so far. However, for the first time the DPP-IV inhibitory activity of *Commiphora mukul* was reported from our laboratory. [21] Results of the present study demonstrated, that the hydroalcoholic extract of *Commiphora mukul* exhibited significant DPP-IV inhibitory effects. *Commiphora mukul* showed comparable DPP-IV inhibitory effects as compared to the marketed synthetic DPP-IV inhibitors; Vildagliptin and Sitagliptin. This could explain the additional pathways with which *Commiphora mukul* could possibly interact to produce its beneficial effects.

Antidiabetic effects of monotherapy and combination therapy as indicated by restoring effect on blood glucose and HbA1c levels was demonstrated in the present study. Observed hypoglycaemic effects of *Commiphora mukul* was in agreement with previous reports by Ramesh *et al* [20] However this is the first report of the synergistic effect of *Commiphora mukul* and metformin combination therapy on reduction of blood glucose levels.

Myocardial salvaging effect in the setting of diabetes was evaluated by several cardiac parameters such as heart to body weight ratio, Myocardial *Creatinine phosphokinase-MB* (CPK-MB) and *B-type* natriuretic peptide (BNP). In addition, histopathological studies were undertaken to confirm the cardioprotective effects in experimental diabetes. The heart to body weight ratio, is considered as an index of cardiac hypertrophy. Boluyt *et al* demonstrated that continuous infusion of ISP in rats elicits typical cardiac gene expression and hypertrophy. [22] The treatment groups were effective in reducing cardiac hypertrophy as compared to the D-ISP Control group. It was observed that MET+VIL combination therapy showed superior reduction in myocardial hypertrophy as compared to MET+CM treated group.

Myocardial necrosis was estimated by measuring the activity of cardiac marker enzymes. During myocardial infarction, enzymes such as CK-MB, and Alanine aminotransferase (ALT) leak out from the myocardial cells into the serum and are therefore well-known diagnostic markers of myocardial infarction.[23] Subsequent to ISP challenge, the serum CPK-MB levels was significantly increased in the D-ISP group as compared to NC group at 5th week of study suggesting the occurrence of considerable membrane damage as compared with the NC group. Previous studies have demonstrated that the CPK-MB released from the myocardium into the blood stream is directly proportional to the number of necrotic cells present in the heart tissue. A myocardial salvaging effect as demonstrated by restoration of myocardial CPK-MB levels was observed in the treatment groups. These results were in accordance with previous clinical and experimental study by Berve and Bhosale *et al* (2014). [24]

Additionally, BNP levels were also significantly reduced in treatment group rats. BNP is secreted predominantly from the ventricles in response to increased wall stress. BNP is known to be one of the major forces driving left ventricular (LV) remodeling. [25] The protective effects of *Commiphora mukul* on myocardial injury demonstrated by biochemical parameters was confirmed by histopathological assessment. Histopathological studies revealed that in combination treatment group rats, occasional focal myofiber loss, less inflammation and edema was observed. However the degree of edema, inflammation and necrosis was less as compared to the monotherapy.

In order to elucidate the possible cardioprotective mechanisms of the natural DPP-IV inhibitors in the setting of diabetes, its effect on multiple targets: myocardial apoptosis, DPP-IV pathway, hyperlipidemia, prothrombotic effects, inflammation and oxidative stress were studied. The precise mechanism for the salutary effects of *Commiphora mukul* in attenuating myocardial infarction co-existing with diabetes will help in better understanding of basic mechanisms of action of these natural DPP-IV inhibitors.

Evidence from several studies has suggested that DPP-IV inhibitors improve cardiac function in both animal and clinical studies. [26] The study by K. A. Connelly *et al* (2013) reported that without affecting glycemic control, DPP-IV 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. [27] In the present study, various treatment protocols resulted in a significant reduction in serum DPP-IV levels as compared to D-ISP group. A superior reduction in serum DPP-IV levels was observed in the combination groups rats as compared to monotherapy. Although the antidiabetic activity of *Commiphora mukul* has been reported, their ability to alter the DPP-IV pathway had not been studied so far.

Interestingly, DPP-IV inhibition correlated with the cardioprotective effects demonstrated by the various treatment groups. Dyslipidemia associated with diabetes may lead to alterations in myocardial enzyme systems, subcellular organelles and myocardial fuel supply and eventually to cardiac disease. [28] Thus alteration in lipid metabolism may be an important determinant of cardiac function in diabetes. In the present study, various treatment protocols showed favorable effects on lipid profile by reducing total cholesterol, triacylglycerol, LDL-C levels and positively modifying HDL-C levels. Significant lipid lowering activity of *Commiphora mukul* has also been well documented in various hyperlipaemic experiment models. [29] Atherogenic index is an useful predictor of cardiovascular risk. Atherogenic index was significantly reduced in monotherapy and combination therapy groups as compared to D-ISP group. Interestingly, the atherogenic index was most favorably restored in MET+CM group.

The pro-thrombotic state is characterized by increased fibrinogen levels, increased plasminogen activator inhibitor and abnormalities in platelet function. It is known from many prospective studies that fibrinogen is a risk factor for cardiovascular disease, including coronary heart disease, stroke, and peripheral arterial disease. [30,31] The results of the present study demonstrated that treatment groups significantly reduced fibrinogen levels as compared to D-ISP treated rats. This is one of the mechanisms contributing to the cardioprotective effects of *Commiphora mukul* in setting of diabetes. Various treatment protocols demonstrated anti-inflammatory activity indicated by significant reduction in inflammatory marker: hs-CRP. Anti-inflammatory activity of medicinal plants may contribute to the stabilization of atherosclerotic plaques and attribute to its cardioprotective effects. Such anti inflammatory activity of *Commiphora mukul* was in accordance with previous study by Szapary *et al* [32]

Oxidative stress causes abnormal gene expression, altered signal transduction, and the activation of pathways leading to programmed myocardial cell death. A significant rise in the level of TBARS was observed in the serum of D-ISP rats, which was decreased significantly after treatment with various treatment protocols. These finding demonstrate that *Commiphora mukul* possess anti-peroxidative effects. However, in the experimental model of MI co-existing with diabetes, there are no reports of synergistic antioxidant effects with metformin combination therapy.

TUNEL positivity was studied to delineate the involvement of apoptosis in the setting of myocardial injury co-existing with diabetes. The progressive loss of cardiomyocytes by apoptosis causes further deterioration of cardiac function. Hence targeting apoptosis appears to be a novel and effective therapeutic strategy for attenuating myocardial injury. [33] In present study enhanced apoptosis as indicated by increased TUNEL positive nuclei was observed in the D-ISP group as compared NC. There are various studies reporting enhanced apoptosis of cardiac myocytes and endothelial cells among diabetic patients as well as in experimental models of diabetes. Additionally apoptosis or programmed cell death, is a common pathological feature in acute MI [34,35] The *Commiphora mukul* protected diabetic rats from myocardial injury through attenuation of myocardial apoptosis.

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. This was attenuated by various treatment protocols and restoring the architecture of the pancreas, liver and kidney. *Commiphora mukul* monotherapy and combination therapy did not adversely affect the pancreatic, liver and kidney function as indicated by pancreatic lipase, SGPT and creatinine levels. Previous reports by Ramesh *et al* [20] concur with the present findings.

The present study for the first generated experimental data on the possible place of natural DPP-IV inhibitors *Commiphora mukul* in antidiabetic therapy by comparing the efficacy with standard antidiabetic drugs. The results of this study showed the superior cardioprotective effects of *Commiphora mukul* combination therapy with Metformin as compared to Metformin monotherapy in setting of diabetes. The rationale for combining metformin with DPP-IV inhibitors is the complimentary mechanism of action. Thus, metformin acts primarily by reducing hepatic glucose output and improving insulin sensitivity in liver and muscle whereas DPP-IV inhibitors act by increasing GLP-1 levels and thereby stimulating insulin secretion and inhibiting glucagon secretion. In addition, metformin has been shown to increase GLP-1 levels [(36)], which would be a potential for an additional synergistic action with DPP-IV inhibitors.

Computational *in silico* studies were used to identify the ingredients of *Commiphora mukul* responsible for DPP-IV inhibition. The active principles of *Commiphora mukul* were computationally designed and screened through *in silico* docking studies against crystal structure of DPP-IV. The *in silico* methods have been demonstrated to be useful in predicting the potential of proteins as precursors of peptides in various bioactivities, such as DPP-IV. The DPP-IV enzyme binding site is highly drug gable in the sense that tight, specific binding to the enzyme can be achieved using small molecules with drug-like physicochemical properties.[37,38] In the present study, the crystal structure of DPP-IV was considered as receptor which was docked against the active ingredients of *Commiphora mukul* (Gluggusterone E, Gluggusterone Z), Sitagliptin and Vildagliptin. The active ingredients of *Commiphora mukul* (Gluggusterone E, Gluggusterone Z) demonstrated significant inhibition of DPP-IV enzyme. The binding energy is inversely proportional to the DPP-IV inhibitory activity. Based on the binding energy results, it was found all the ligands studied had superior DPP-IV binding affinity as compared to Sitagliptin. Gluggusterone E, Gluggusterone Z and Vildagliptin prefer to bind within the active site pocket (the 1st largest pocket). But Sitagliptin prefers to bind in the second largest pocket.

The present study has demonstrated the DPP-IV inhibitory activity and cardioprotective efficacy of medicinal plants *Commiphora mukul* in setting of diabetes. There may be several mechanisms (DPP-IV inhibition, hypolipidemic, reduced artherogenic potential, anti-thrombotic state, anti-inflammatory, antioxidant) contributing to its beneficial cardioprotective effects. The highlight of the study is that the myocardial salvaging effects of *Commiphora mukul* + Metformin were found to be comparable to Vildagliptin + Metformin combination therapy. Interestingly, combination therapy was found to be superior compared to Metformin monotherapy. Thus, results demonstrate the cardiovascular beneficial effects of *Commiphora mukul* adjuvant therapy along with standard drug Metformin in myocardial infarction co-existing with diabetes mellitus.

DPP-IV based therapeutics may represent novel targets, the cardioprotective actions of which may translate into demonstrable therapeutic benefits in diabetes with co-existing cardiovascular diseases. However, further studies are needed to confirm these finding and establish the therapeutic beneficial effects of *Commiphora mukul* in standard antidiabetic therapy.

5. Conclusion

Commiphora mukul possesses significant myocardial salvaging effects in the setting of diabetes mellitus. Cardioprotective effects may be attributes to DPP-IV inhibitory activity, hypolipidemic, reduced atherogenic potential, anti-thrombotic state, anti-inflammatory, antioxidant and anti-apoptotic activities of *Commiphora mukul* which was found to be comparable to synthetic DPP-IV Inhibitor: Vildagliptin. Based on in silico studies, it was found that the active ingredient of *Commiphora mukul* such as Gluggusterone E, Gluggusterone Z possesses significant DPP-IV inhibitory activity as delineated, using *in silico* docking suggesting potential benefits of developing these active ingredients as natural DPP-IV inhibitors.

Acknowledgment

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Reference

[1]. UK Prospective Diabetes Study (UKPDS) Group. *Lancet*.1998; 352:837-855.

[2]. Angelo Avogaro, Saula de Kreutzenberg and Gianpaolo Fadini. Dipeptidyl-peptidase 4 Inhibition: Linking Metabolic Control to Cardiovascular Protection. *Current Pharmaceutical Design*. 2014; 20:2387-2394.

[3]. Scheen AJ. Cardiovascular effects of dipeptidyl peptidase-4 inhibitors: from risk factors to clinical outcomes. *Postgrad Med.*2013; 125(3):7-20.

[4]. Fisman and Tenenbaum Antidiabetic treatment with gliptins: focus on cardiovascular effects and outcomes. *Cardiovascular Diabetol*. 2015; 14(129):1-13.

[5]. Matteucci E, Giampietro O. Dipeptidyl peptidase-4 inhibition: linking chemical properties to clinical safety. *Curr Med Chem.* 2011; 18(31):4753-4760.

[6]. Nicolau Ramis J, Masmiquel Comas L. The new antidiabetic Agents in firing line. safety reason or witch hunt. *Endocrinol Nutr*. 2012; 59(1):1-8.

[7]. Monami M, Dicembrini I, Mannucci E. Dipeptidyl peptidase-4 inhibitors and pancreatitis risk: a meta-analysis of randomized clinical trials. *Diabetes, Obesity & Metabolism*. 2014; 16:48-56.

[8]. Elashoff M, Matveyenko A. V., Gier B, Elashoff R, and Butler P C. Pancreatitis and Thyroid Cancer with Glucagon like Peptide-1-based Therapies. *Gasteroenterology* 2011; 141:150-156.

[9]. Kalshetti P, Thakurdesai P, Alluri R. A Review on Bioactive Phytoconstituents and Pharmacological Uses of *Commiphora mukul*. *J Curr Pharma Res*. 2014; 5(1): 1392-405.

[10]. Widad M. Al- Bishri and Omar S. Al-Attas. Guggul Resin Extract Improve hyperglycemia and Lipid Profile in Streptozotocin Induced Diabetes Mellitus in rats. *Life Sci J*. 2013; 10(1): 2735-2741.

[11]. Ojha S, Bhatia J, Arora S, Golechha M, Kumari S, Arya DS. Cardioprotective effects of Commiphora mukul against isoprenaline-induced cardiotoxicity: A biochemical and histopathological evaluation. *Journal of Environmental Biology*. 2011; 32(6):731-738.

[12]. Rakieten, N., Rakieten, M.L., Nadkarni, M.V. Studies on the diabetogenic action of streptozotocin. *Cancer Chemother. Rep*.1963; 29:91-880.

[13]. Al-masri, I.M., Mohammad, M.K., Tahaa, M.O. Inhibition of dipeptidyl peptidase IV (DPP-IV) is one of the mechanisms explaining the hypoglycemic effect of berberine. *J. Enzyme Inhib. Med. Chem.* 2009; 24(50):1061-1066.

[14]. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE. The protein data bank. *Nucleic Acids Research*. 2000; 28(1):235-42.

[15]. Kim, S., Thiessen, P.A., Bolton, E.E., Bryant, S.H. PubChem substance and compound databases. *Nucleic Acids Res*. 2016; 4(44): 1202-1213.

[16]. Leite TB, Gomes D, Miteva MA, Chomilier J, Villoutreix BO, Tufféry P. Frog: a FRee Online druG 3D conformation generator. Nucleic acids research. 2007; 35(suppl_2): W568-72.

[17]. Dundas, J., Ouyang, Z., Tseng, J., Liang, J. CASTp: computed at as of surface topography of proteins with structural and topographical mapping of functionally annotated residues. *Nucleic Acids Res*. 2006; 34: 116-118.

[18]. Ritchie, D., Kozakov, D., Vajda, S. Accelerating and focusing protein-protein docking correlations using multi-dimensional rotational FFT generating functions. *Bioinformatics.* 2008; 24: 1865-1873.

[19]. Guex, N., Peitsch, M.C. SWISS-MODEL and the Swiss Pdb Viewer: an environment for comparative protein modeling. *Electrophoresis*.1997; 18:2714-2723.

[20]. Bellamkonda R, Rasineni K, Singareddy SR, Kasetti RB, Pasurla R, Chippada AR, Desireddy S. Antihyperglycemic and antioxidant activities of alcoholic extract of Commiphora mukul gum resin in streptozotocin induced diabetic rats. *Pathophysiology.* 2011; 18(4):255-61.

[21]. Borde, M.K., Mohanty, I.R., Maheshwari, U., Suman, R., Deshmukh, Y.A. Dipeptidylpeptidase –IV Inhibitory activites of medicinal plants: *terminalia arjuna*, *Commiphora mukul*, *gymnema sylvestre*, *morinda citrifolia* and *emblica officinalis*. *Asian J. Pharm. Clin. Res*. 2016; 9 (3): 180-182.

[22]. Boluyt MO, Long X, Eschenhagen T, Mende U, Schmitz W, Crow MT, and Lakatta EG. Isoproterenol infusion induces alterations in expression of hypertrophy-associated genes in rat heart. *Am J Physiol*.1995; 269:638-647.

[23]. Murugesan M, Ragunath M, Nadanasabapathy S, Revathi R and Manju, V. Protective role of fenugreek on isoproterenol induced myocardial infarction in rats. *International Research Journal of Pharmacy*. 2012; 3(2): 2.

[24]. Kalyani Barve and Nima Bhonsle. Commiphora mukul Prevents Myocardial Dysfunction in Streptozotocin Induced Diabetic Rats. *Pharmaceutical Crops*. 2014; 5(5):61-66.

[25]. Güneş Y, Okçün B, Kavlak E, Erbaş C, and Karcier S, Value of brain natriuretic peptide after acute myocardial infarction, *Anadolu. Kardiyol. Derg*. 2008; 8(3):182-187.

[26]. Read PA, Khan FZ, Dutka DP. Cardioprotection against ischaemia induced by dobutamine stress using

glucagon-like peptide-1 in patients with coronary artery disease. *Heart*. 2012; 98:408-413.

[27]. Kim Alexander Connelly, Yanling Zhang, Andrew Advani, Suzanne L. Advani, Kerri Thai, Darren A. Yuen & Richard E. Gilbert DPP-4 Inhibition Attenuates Cardiac Dysfunction and Adverse Remodeling Following Myocardial Infarction in Rats with Experimental Diabetes. *Cardiovascular Therapeutics*. 2013; 31:259-267.

[28]. Rodrigues BH, Xiang, Mc Neill JH. Effect of Lcarnitine treatment on lipid metabolism and cardiac performance in chronically diabetic rats. *Diabetes.*1988; 37:1358-1364.

[29]. Sharma, B., R. Salunke, S. Srivastava, C. Majumder and P. Roy. Effects of guggulsterone isolated from Commiphora mukul in high fat diet induced diabetic rats. *Food Chem. Toxicol*.2009; 47:2631-2639.

[30]. Byberg L, Siegbahn A, Berglund L, McKeigue P, Reneland R, Lithell H .Plasminogen activator inhibitor-1 activity is independently related to both insulin sensitivity and serum triglycerides in 70-year- old men. *Arterioscler. Thromb. Vasc. Biol.*1998; 18:258-264.

[31]. Trovati M, Anfossi G. Insulin, insulin resistance and platelet function: similarities with insulin effects on cultured vascular smooth muscle cells. *Diabetologia.*1998; 41: 609-622.

[32]. Philippe O. Szapary, Megan L. Wolfe, LeAnne T. Bloedon, RD; Andrew J. Cucchiara, Ara H. DerMarderosian, Michael D. Cirigliano, Daniel J. Rader Guggulipid for the Treatment of Hypercholesterolemia A Randomized Controlled Trial *JAMA*.2003;290-296.

[33]. Quanzhou Feng, Shiwen Wang Cardiomyocytic apoptosis and heart failure of Geriatric Cardiology *Journal Clinical Research*. 2008;(15): 21-25

[34]. Cai L, Li W, Wang G, Guo L, Jiang Y, Kang YJ. Hyperglycemia induced apoptosis in mouse myocardium: mitochondrial cytochrome C-mediated caspase-3 activation pathway. *Diabetes.* 2002; 51:1938-1948.

[35]. Abbate A, Bussani R, Amin MS, Vetrovec GW, Baldi A. Acute myocardial infarction and heart failure: role of apoptosis. *Int J Biochem Cell Biol*.2006; 38:1834-1840.

[36]. Mannucci E, Ognibene A, Cremasco F, *et al*. Effect of metformin on glucagon-like peptide 1 (GLP-1) and leptin levels in obese nondiabetic subjects. *Diabetes Care.* 2001; 24:489-494.

[37]. Kuhn B, Hennig M, Mattei P. Molecular recognition of ligands in dipeptidyl peptidase IV. *Current Topics in Medicinal Chemistry.*2007; 7(6):609-619.

[38]. Zettl H, Schubert-Zsilavecz Manfred, Steinhilber D. Medicinal chemistry of incretin mimetics and DPP-4 inhibitors. *Chem Med Chem*. 2010; 5(2):179-185.