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# Preclinical investigation of role of an ayurvedic preparation, Pramehari ark in type-2 diabetes

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**Abstract**
Many classical drugs are claimed to have hypoglycemic activity that make them valuable for people with or at high risk of type 2 diabetes. Vijaysar (*Pterocarpus marsupium*) and Gaumutra (Indian cow urine) both have shown antidiabetic property since classical time and both shows synergistic effect in combination named as Pramehari ark. In this study, the Type-2 diabetes was induced by high-fat diet and low-dose streptozotocin injection in rats which is characterized by increasing growth rate/body weight, histological changes in the heart and biochemical parameters. It was shown that all of the high-fat diet plus STZ injection rats exhibited remarkable lesions and plaque representing damage to heart. Rats suffered from hyperglycemia during whole study duration. The profiling of biochemical parameters indicated that blood glucose, lipids, serum parameters and urine albumin, creatinine etc were much higher in high-fat diet plus STZ injection rats than that in controls. The findings demonstrated that Pramehari ark prevented the pathological progression of type 2 diabetes in rats.
**Keywords**: Gaumutra, High fat diet, Hypoglycemic activity, Pramehari ark, Streptozotocin, Vijaysar

## 1. Introduction

Currently Diabetes mellitus (DM) is a major universal health issue. Various environmental factors and hereditary interaction results into a chronic metabolic disorder(Sheela, Jose et al. 2013). It is significantly affecting social, psychological and physical aspects of life(Khanra, Dewanjee et al. 2015). Around 366 million individuals are suffering from DM around the globe. By 2030 the incidence of this disease is predicted to be more than double (Fernández-Millán, Ramos et al. 2014).
Type 2 diabetes mellitus (T2DM) is a metabolic disorder characterized by chronic low-grade inflammation, poor insulin sensitivity, and impaired insulin secretion (Hameed, Masoodi et al. 2015). It has been suggested that regular physical activity may prevent or delay diabetes and its complications(Alaca, Uslu et al. 2018).Pathogenesis of diabetes includes generation of free radicals and the increased levels of blood glucose are associated with high lipid peroxidation (LPO), which may contribute to long-term tissue damage(Aboonabi, Rahmat et al. 2014).
From last two decades keen interest is shown by the researchers in variety of natural products with antioxidant potential which can play a major role in protecting against the molecular damage induced by reactive oxygen species (ROS)(MP, Joshi et al. 2010).
The present study includes cow urine aimed at evaluating its anti-diabetic potential. The sacred Indian cow, Bos indicus, is believed to be a "mobile hospital" for the treatment of many diseases. Cow urine is described in detail in ancient Classical scriptures, such as Charaka samhita, Shushruta samhita and Brahad-Wagbhatt etc.(Chauhan 2004).
*Pterocarpus marsupium Roxb.* (Leguminosae) another constituent of the preparation, vernacularly called Vijaysar has been reported to contain flavonoids, mucilage, glycosides, saponin, tannin and epicatechin (Verma, Kamboj et al.). The heart wood of *Pterocarpus marsupium*has been used in ancient medicine as antibacterial, antidiabetic and antileprotic (Chopra, Chopra et al. 1958). Due these medicinal properties, the present study was designed to investigate the antidiabetic, antihyperlipidemic and antioxidant activity of cow urine extract of *Pterocarpus marsupium* wood in streptozotocin induced diabetic rats(Vijayan and Sibi).Further, although Indian Ayurvedic literature cites various medicinal properties of cow urine, there is very little scientific evidence to support this. Hence, the present study was undertaken to provide the effect of combination of cow urine and Vijaysar.

## 2. Material and Method

### 2.1 Collection, identification and authentication of materials

The heart wood of the plant was collected from the outfield of Rajpipla, Gujarat, India and it was identified and authenticated by Department of Biosciences, Saurashtra University, Rajkot, Gujarat (SU/BIO/972). Urine of Bos indicus cow was collected from Kamdhenu Gaushala, Jamnagar, Gujarat.

### 2.2 Preparation of the formulation

For preparation of an extract, 100 g of powder of heart wood of *Pterocarpus marsupium was* soaked overnight in 1-liter Cow urine which is freshly collected. Then the formulation was prepared by distillation method. The formulation is classically named as Pramehari ark (PA). *Pterocarpus marsupium* Water extract was also used to compare the effect of Cow urine. Water extract was

prepared by soaking 100 g of powder of heart wood of *Pterocarpus marsupium* in 1-liter water.Then the extract was prepared by distillation method.

### 2.3 Animals

Animal models comprised of female Wistar rats (weight 200 ± 20 g; age 2–3 months). The rats were housed in standard polypropylene cages (47 cm × 34 cm × 20 cm) under standard lab conditions of 12 h light–dark cycle, temperature (25 ± 2°C), relative humidity (50 ± 15%), standard diet (Baramati Agro ltd, Baramati) and water ad libitum. The animal experiments were pursued at the Department of Pharmaceutical Sciences, Saurashtra University, Rajkot, Gujarat, India (SU/DPS/IAES/2012/1217). The experiment was carried out according to the guidelines of the committee for the purpose of control and supervision of experiments on animals (CPCSEA), New Delhi, India.

### 2.4 Induction of Diabetes

The female Wistar-albino rats (200-250 g, age 2–3 months) were randomly divided into 6 groups: Normal control (NC, n=8), Diabetic control (DC, n=8), Diabetic treated with standard drug Glipizide(5 mg/kg) (Std, n=8), Lower dose of treatment(PA 400 mg/kg) (Test-1, n=8), Higher dose of treatment (PA 800 mg/kg) (Test-2, n=8), Water extract of *Pterocarpus marsupium*(800 mg/kg) (WE, n=8). Control rats were fed with standard rat chow. Diabetic rats in untreated group and treated group were fed with high-fat chow (ingredients: 10% refined lard, 20% sucrose, 2% cholesterol, 1% sodium cholate and 67% common food)(Guo, Qin et al. 2012)[19]. Four weeks later, rats in disease control group and treated group were given the peritoneal injection of a low dose of streptozotocin (35 mg/kg body weight; Sigma, St. Louis, USA) dissolved in citrate buffer (0.1M, pH- 4.5), while the normal control group was given equivalent volume of distilled water. After 1 week, animals exhibiting fasting glucose levels between 140–200 mg/dl were screened as type 2 diabetic (T2D) ratsand were used for the antidiabetic assay. Formulation was administered to rats orally using an intragastric tube for the period of 60 days. Fatty food was continued till the end of the study.

## 3. Serum Biochemical Analysis

For estimation of serum biochemical parameters, Eppendorf tubes were used for collection of blood samples after 60 days. From this blood, serum was separated by centrifugation at 10000 rpm for 15 min and stored at $-20^0$C until the analysis was carried out(Dewanjee, Das et al. 2009). Enzymatic colorimetric kits (Span Diagnostics Ltd., Surat, India) were used for determination of serum triglyceride and cholesterol profiles following manufacturers protocols. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST), urea and the membrane leakage enzymes (creatine kinase and lactate dehydrogenase) were also assessed by standard kits (Span Diagnostic Limited, India)(Nayak and Pattabiraman 1981)

For the measurement of urine parameters, animals were placed in metabolic cages with free access to water and food and urine was collected for 24 hours in a clean, dry beaker and filtered to remove turbidity. C-reactive protein (CRP), Lactate Dehydrogenase (LDH), Creatinine Kinase (CK), Creatinine, Blood urea (BU), Albumin were checked spectrophotometrically (Star 21plus, Aspen diagnostics Ltd., Italy) using available biochemical diagnostic kits (Agappe Diagnostics Pvt. Ltd., India).

### 3.1 Organs' biochemical analysis

The rats were anesthetized and sacrificed by cervical dislocation after 8 weeks. The organs (heart and kidney) were excised, cleaned and washed with ice-cold saline (pH 7.4). The organs were homogenized in Tris–HCl (0.1 M)-EDTA buffer (pH 7.4, 0.001 M) and centrifuged at12,000 g for 30 min at 4°C. Biochemical parameters were assessed from collected supernatant.
The extent of lipid peroxidation was measured by evaluating thiobarbituric acid reactive substances (TBARS) following the protocol of Ohkawa et al.(Ohkawa, Ohishi et al. 1979). Reduced glutathione (GSH) level was measured by Hissin and Hilf's method (Hilf and Hissin 1976). Activity of antioxidant enzymes like catalase (CAT), superoxide dismutase (SOD) was estimated by the methods reported by Ghosh et al. (Ghosh, Das et al. 2010).

### 3.2 Histopathology

Isolated organs from normal and all diseased rats were fixed in buffered formalin (10%) and processed for paraffin sectioning. Sections obtained (approx. 5 μm) were stained by hematoxylin and eosin to study the histology of isolated organs.

### 3.3 Statistical analysis

One-way ANOVA was utilized for statistical analysis of data and expressed as mean ± SE followed by Dunnett's t-test using computerized Graph Pad Prism 5.0 (Graph Pad Software, San Diego, CA, USA). The values were considered significant when $p < 0.05$.

## 4. Results

### 4.1 Estimation of body weight

At the end of 8 weeks treatment, streptozotocin (STZ) treated rats exhibited significantly decreased levels of body weight compared to control rats. Treatment with the test drugs showed significant marked increase in body weight as compared to diabetic control rats and treated with water extract rats.

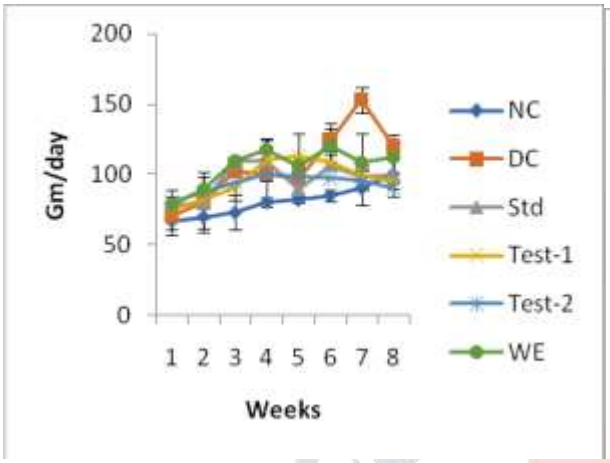
**Table 1 Effect of the test drugs on body weight of diabetic rats.**

| Parameter | NC | DC | Std | Test-1 | Test-2 | WE |
|---|---|---|---|---|---|---|
| **Body weight** | 222.4± 1.315 | 198.3± 1.315*** | 231.6± 2.51*** | 228.7± 1.287*** | 235.38 ± 1.056*** | 220.78± 1.023*** |

Values are expressed as Mean ± SEM (n=6), statistically analysis was performed by One way ANOVA, followed by multiple comparison Dunnett test's multiple range tests. NC–Normal control, DC–Diabetic control, Std- Diabetic treated with glipizide (5 mg/kg, PO) Test-1-Diabetic treated with the formulation (400 mg/kg, PO), Test-2-Diabetic treated with the formulation (800 mg/kg, PO), WE-Diabetic treated with water extract of vijaysar (800 mg/kg, PO).Normal group is compared with disease control group, disease control group is compared with all the treatment groups, $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$.

### 4.2 Estimation of food and water intake

In our study food and water intake was measured throughout the study. STZ treated rats showed increased food and water intake and weight loss. After the treatment with Pramehari ark significant restoration of the body weight and decrease in the food and water intake was observed.



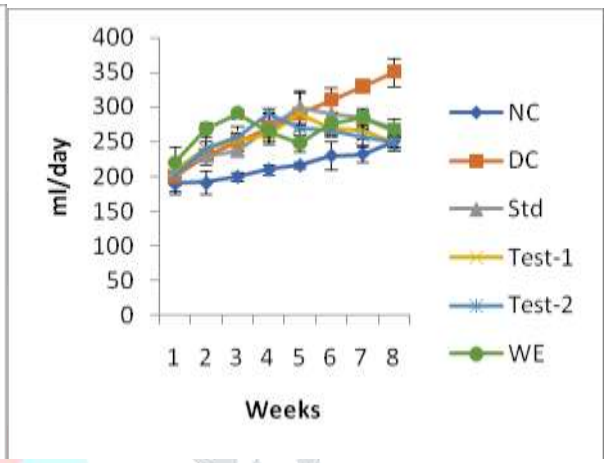




**Fig.1 Estimation of food intake Fig.2 Estimation of water intake**

### 4.3Estimation serum glucose

At the end of 8 weeks treatment, streptozotocin (STZ) treated rats exhibited significantly ($p < 0.001$) increased levels of serum glucose compared to control rats. Treatment with the formulation showed significant ($p < 0.001$) reduction in glucose levels as compared to diabetic control rats (Table 2). Treatment with 400 mg/kg dose of the formulation also show significant decrease in serum glucose as compared to diabetic rats but water extract shows somewhat lower effect than combination.

**Table 2 Effect of the formulation on serum glucose in diabetic rats.**

| Parameter | NC | DC | Std | Test-1 | Test-2 | WE |
|---|---|---|---|---|---|---|
| **Glucose (mg/dl)** | 92.69± 2.168 | 353.9± 26.45*** | 118.2± 11.84*** | 170.2± 9.37*** | 93.68± 1.744*** | 152.5± 8.748** |

Values are expressed as Mean ± SEM (n=6), statistically analysis was performed by One way ANOVA, followed by multiple comparison Dunnett test's multiple range tests. NC–Normal control, DC–Diabetic control, Std- Diabetic treated with glipizide (5 mg/kg, PO) Test-1-Diabetic treated with the formulation (400 mg/kg, PO), Test-2-Diabetic treated with the formulation (800 mg/kg, PO), WE-Diabetic treated with water extract of vijaysar (800 mg/kg, PO).Normal group is compared with disease control group, disease control group is compared with all the treatment groups, $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$.

### 4.4 Serum lipid profile markers

**Effect of Pramehari ark on serum triglyceride, cholesterol, and low density Lipoprotein and high density lipoprotein.**

At the end of 8 weeks treatment, Serum TG, T-CHO and LDL-C levels of the diabetics groups were significantly ($p < 0.001$) increased, while HDL-C was decreased compared to the control group rats. Treatment with the formulation showed significant ($p < 0.001$) reduction in Serum TG, T-CHO and LDL-C levels (Table 3) and significantly increased HDL-C level in as compared to diabetic control rats (Table 3).

**Table 3 Effect of Pramehari ark on serum triglyceride, cholesterol, low density lipoprotein and high density lipZoprotein.**

| Parameters | Control | Disease control | Standard | Test-1 | Test-2 | WE |
|---|---|---|---|---|---|---|
| **TC (mg/dl)** | 53.43 ±5.115 | 154.0 ±10.12*** | 70.26 ±4.930*** | 124.4 ±6.043** | 74.92 ±4.143*** | 120.1 ±9.707** |
| **TG (mg/dl)** | 64.14 ±3.106 | 127.3 ±4.170*** | 72.40 ±2.035*** | 100.1 ±1.551** | 73.74 ±2.863*** | 106.5 ±2.563** |
| **LDL (mg/dl)** | 27.96 ±0.9019 | 124.8 ±2.701*** | 33.85 ±2.833*** | 67.33 ±1.714*** | 41.52 ±3.292*** | 79.69 ±2.296*** |
| **HDL (mg/dl)** | 33.22 ±3.517 | 11.2 ±4.670*** | 26.74 ±2.708** | 22.1 ±4.679* | 28.17 ±2.599** | 18.99 ±3.359* |

Values are expressed as Mean ± SEM (n=6), statistically analysis was performed by One way ANOVA, followed by multiple comparison Dunnett test's multiple range tests. NC–Normal control, DC–Diabetic control, Std- Diabetic treated with glipizide (5 mg/kg, PO) Test-1-Diabetic treated with the formulation (400 mg/kg, PO), Test-2-Diabetic treated with the formulation (800 mg/kg, PO), WE-Diabetic treated with water extract of vijaysar (800 mg/kg, PO).Normal group is compared with disease control group, disease control group is compared with all the treatment groups, $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$.

### 4.5 Oxidative parameter of Heart tissue

**Effect of Pramehari ark on malondialdehyde, superoxide dismutase and glutathione in heart tissue.**

At the end of 8 weeks treatment, the level of MDA in the heart was significantly ($p < 0.001$) increased in untreated diabetic rats compared with the rats in control group. Test drug treatment significantly ($p < 0.001$) reduced the MDA level as compared to untreated diabetic group in cardiac tissue. In the untreated diabetic group, Collagen levels was significantly ($p < 0.001$) higher and decreased ($p < 0.05$), ($p < 0.01$) activates of GSH and SOD than the control and diabetic treated group rats. Additionally, Collagen level was significantly decreased in the formulation treated diabetic rats compared with untreated diabetic rats (Table 25 & Fig. 19,20,21). However, GSH and SOD activities, an activity were increased ($p < 0.01$), ($p < 0.05$) in formulation treated group rats as compared with untreated diabetic rats (Table 7).

**Table 4 Effect of Pramehari ark on malondialdehyde, superoxide dismutase and glutathione in heart tissue.**

| Parameters | NC | DC | Std | Test-1 | Test-2 | WE |
|---|---|---|---|---|---|---|
| **MDA (nmoles/mg protein)** | 0.088 ± 0.002 | 0.370 ± 0.018*** | 0.240 ± 0.141*** | 0.210 ± 0.016*** | 0.508 ± 0.180*** | 0.171 ± 0.008** |
| **SOD (U/mg protein)** | 183.9± 13.05 | 35.84± 2.905*** | 177.8± 10.28*** | 92.74± 3.193*** | 174.8± 5.619*** | 72.28± 2.227** |
| **GSH (µg/mg protein)** | 0.890± 0.021 | 0.311± 0.013*** | 0.738± 0.026*** | 0.461± 0.027** | 0.788± 0.041*** | 0.391± 0.037** |

Values are expressed as Mean ± SEM (n=6), statistically analysis was performed by One way ANOVA, followed by multiple comparison Dunnett test's multiple range tests. NC–Normal control, DC–Diabetic control, Std- Diabetic treated with glipizide (5 mg/kg, PO) Test-1-Diabetic treated with the formulation (400 mg/kg, PO), Test-2-Diabetic treated with the formulation (800 mg/kg, PO), WE-Diabetic treated with water extract of vijaysar (800 mg/kg, PO).Normal group is compared with disease control group, disease control group is compared with all the treatment groups, $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$.

### 4.6 Histopathology of heart tissue

At the end of 8 weeks treatment, the hearts of control rats have a normal morphology with abundant muscle fibers and scarce connective tissue. The muscle fibers were made up of cellular units joined end to end in wide networks and they had abundant cytoplasm. The connective tissue was thin and well highlighted. Degenerative changes were evident in STZ-induced diabetic rats. The muscle fibers were disorganized and the network disappeared. The connective tissue was clearly increased and the fibrosis was evident around these cardiac fibers. The animals treated with Pramehari ark showed a morphological pattern similarly to that of controls and showed very little amount of collagen distributed throughout anatomical structures.

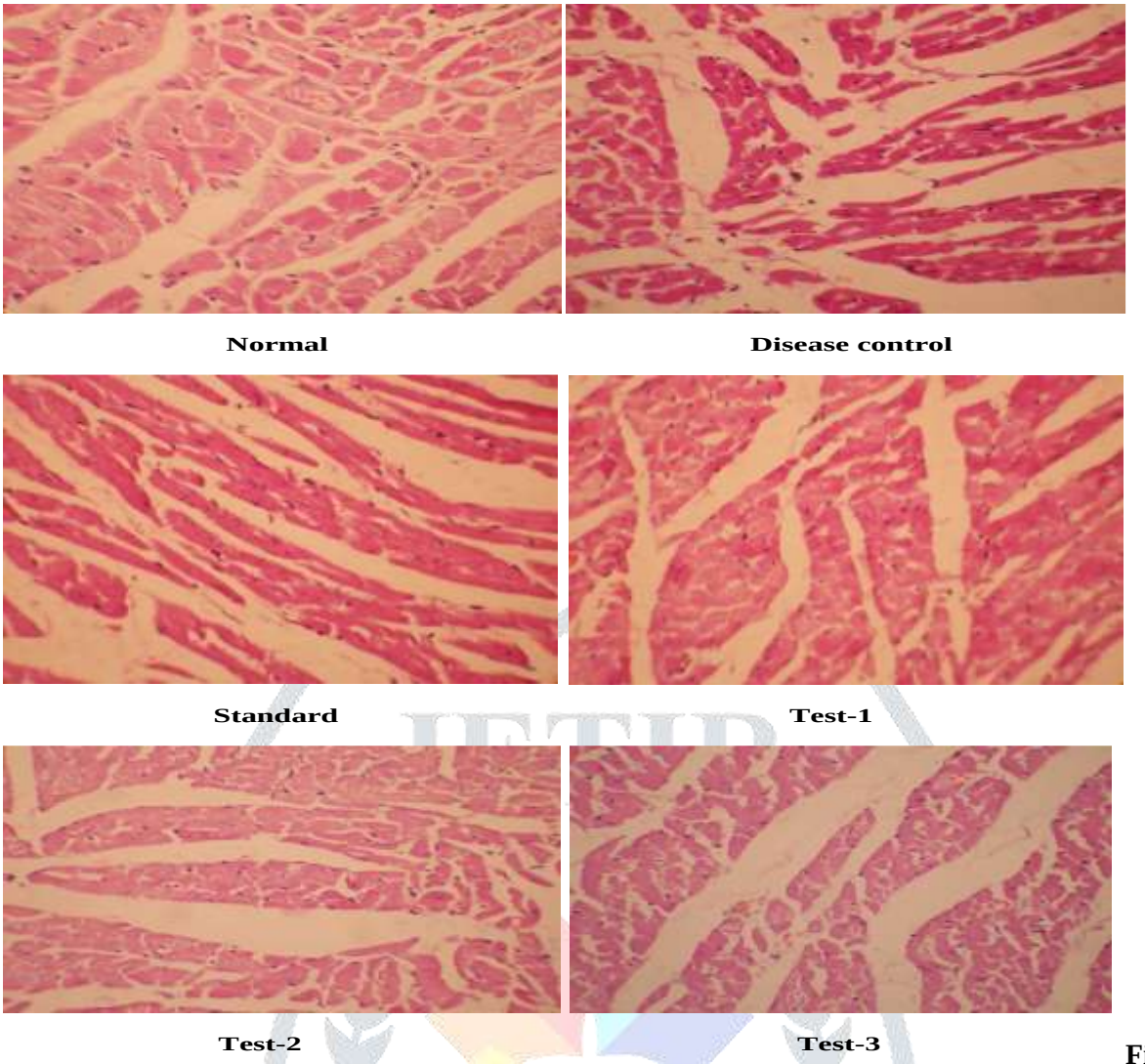



**Fig.2**

**Histopathology of heart tissue**

## 5. Discussion

The study was intended to develop an ideal model for type 2 diabetes which closely resemblance to metabolic characteristics of clinical type 2 diabetes. The search has investigated the effect of Pramehari ark in High fat diet(HFD) and STZ induced Type-2 diabetes in rats(Srinivasan, Viswanad et al. 2005).

On account of HFD, insulin resistant rats were produced over a short period of time. After 4 weeks of HFD rats were found increased body weight and mild hyperglycemia, a condition resembling to Prediabetic(Tan, Tan et al. 2005).STZ selectively diminishes pancreatic β cells by damaging DNA and causes methylation of DNA, which activates poly(ADP-ribose) polymerase (PARP). As a result, there is depletion of cellular NAD+, reduction in ATP and conclusivelycell death (Bolzán and Bianchi, 2002). The rats treated with PA showed antidiabetic activity against HFD and STZ induced diabetes. But the higher dose of PA shows more significant results than the lower dose and WE.

Dyslipidemia is an essential accompaniment of diabetes which represents high risk factor for coronary heart diseases (Yadav, Vats et al. 2002). One of the major pathogenesis of lipid metabolism disturbances in diabetes is the increased mobilization of fatty acids from adipose tissue and secondary elevation of free fatty acid level in the blood. The consequences of excessive mobilization of fatty acid is the production of ketone bodies in the liver (Singh, Palma et al. 1987). Excessive lipolysis has been found to occur during diabetes. The excessive lipolysis in diabetic adipose tissue leads to increase free fatty acids in circulation. They enter the liver and are esterified to form triglycerides(Palaian, Chhetri et al. 2005). Diabetes is also known to be associated with an increase in the synthesis of cholesterol, which may be due to the increased activity of HMG Co A reductase.A number of observations indicate that plasma HDL cholesterol is low in untreated insulin-deficient diabetics (Glasgow, August et al. 1981). In present study, STZ diabetic rats showed significant increase in total cholesterol, LDL and triglycerides levels and significant decrease in HDL. Chronic treatment with PA significantly reduced elevated total cholesterol, LDL and triglycerides(TG) but did not significantly increased HDL.

Increased oxidative damage and prooxidant as well as defects in antioxidants defense systems could be related to the complications in diabetes patients(Mohlke, Jackson et al. 2005). Various researches also suggested that oxidative stress occurs via non-enzymatic glycation, glucose autoxidation, and alterations in polyol pathway in diabetes patients of all types and is implicated in the pathogenesis of diabetic complications(Jones, Franco et al. 2015).During chronic diabetes, the physiological response to confront oxidative stress is overwhelmed, resulting in an imbalance between prooxidative and anti-oxidative compounds. Results from in vitro experiments have reported that the generation of reactive oxygen species(ROS) is likely to be involved in inducing abnormal responses to hyperglycaemia (Asbun and Villarreal 2006). Also, activation of iNOS contributed to left ventricular contractile dysfunction (Song et al., 2008). In the present study, we found a significant increase in pro-oxidant malondialdehyde (MDA) and NO levels in LV and decrease in antioxidant enzyme activity like superoxide dismutase(SOD) and reduced

glutathione levels in LV in diabetic rats. Chronic treatment with PA showed significant decrease in prooxidant and significant increase in antioxidant enzyme levels in diabetic rat hearts. There are multiple sources of oxidative stress in diabetes including nonenzymatic, enzymatic and mitochondrial pathways. Hyperglycemia can directly cause increased ROS generation. In hyperglycemia, there is enhanced metabolism of glucose through the polyol (sorbitol) pathway, which also results in enhanced production of superoxide radical. Glucose can undergo autoxidation and generate hydroxyl radicals (Turko, Marcondes et al. 2001). In addition, glucose reacts with proteins in a nonenzymatic manner.

## 6. Conclusion

Concluding our findings, the present study provides strong evidence in establishing antidiabetic potential of Pramehari ark, which includes decreased levels of cardiac and renal markers and also includes raising activity of protective antioxidants. The study illustrates that test drugs, seems to be highly promising agents in protecting Type-2 diabetes as well as complication of diabetes. However, further studies are required to confirm this finding and reveal the exact mechanism of action before clinical applications.

## 7. Acknowledgement

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## 8. References

1. Aboonabi, A., A. Rahmat and F. Othman (2014). "Antioxidant effect of pomegranate against streptozotocin-nicotinamide generated oxidative stress induced diabetic rats." Toxicology reports**1**: 915-922.
2. Alaca, N., S. Uslu, G. Gulec Suyen, U. Ince, M. Serteser and H. Kurtel (2018). "Effects of different aerobic exercise frequencies on streptozotocin–nicotinamide-induced type 2 diabetic rats: Continuous versus short bouts and weekend warrior exercises: 不同有氧运动频率对链脲霉素-烟酰胺诱导的 2 型糖尿病大鼠的影响: 持续运动与短时间运动以及周末运动的对照研究." Journal of diabetes**10**(1): 73-84.
3. Asbun, J. and F. J. Villarreal (2006). "The pathogenesis of myocardial fibrosis in the setting of diabetic cardiomyopathy." Journal of the American College of Cardiology**47**(4): 693-700.
4. Chauhan, R. (2004). "Panchgavya Therapy (Cowpathy): Current status and future directions." The Indian Cow: The Scientific and Economic Journal**1**(1): 3-7.
5. Chopra, R., I. Chopra, K. Handa and L. Kapur (1958). "Indigenous drugs of India, UN Dhur and sons Pvt." Ltd., Calcutta**289**: 665.
6. Dewanjee, S., A. K. Das, R. Sahu and M. Gangopadhyay (2009). "Antidiabetic activity of Diospyros peregrina fruit: effect on hyperglycemia, hyperlipidemia and augmented oxidative stress in experimental type 2 diabetes." Food and chemical toxicology**47**(10): 2679-2685.
7. Fernández-Millán, E., S. Ramos, C. Alvarez, L. Bravo, L. Goya and M. Á. Martín (2014). "Microbial phenolic metabolites improve glucose-stimulated insulin secretion and protect pancreatic beta cells against tert-butyl hydroperoxide-induced toxicity via ERKs and PKC pathways." Food and chemical toxicology**66**: 245-253.
8. Ghosh, J., J. Das, P. Manna and P. C. Sil (2010). "Protective effect of the fruits of Terminalia arjuna against cadmium-induced oxidant stress and hepatic cell injury via MAPK activation and mitochondria dependent pathway." Food chemistry**123**(4): 1062-1075.
9. Giunti, S., G. Bruno, M. Veglio, G. Gruden, D. J. Webb, S. Livingstone, N. Chaturvedi, J. H. Fuller and P. C. Perin (2005). "Electrocardiographic left ventricular hypertrophy in type 1 diabetes: prevalence and relation to coronary heart disease and cardiovascular risk factors: the Eurodiab IDDM Complications Study." Diabetes Care**28**(9): 2255-2257.
10. Glasgow, A. M., G. P. August and W. Hung (1981). "Relationships between control and serum lipids in juvenile-onset diabetes." Diabetes Care**4**(1): 76-80.
11. Guo, Z., Z. Qin, R. Zhang, J. Li and Y. Yin (2012). "Effect of rosiglitazone on the expression of cardiac adiponectin receptors and NADPH oxidase in type 2 diabetic rats." European journal of pharmacology**685**(1-3): 116-125.
12. Hameed, I., S. R. Masoodi, S. A. Mir, M. Nabi, K. Ghazanfar and B. A. Ganai (2015). "Type 2 diabetes mellitus: From a metabolic disorder to an inflammatory condition." World journal of diabetes**6**(4): 598.
13. Hilf, R. and P. Hissin (1976). "A fluorometric method for determination of oxidized and reduced glutathione in tissues." Analytical biochemistry**74**(1): 214-226.
14. Jones, S. P., N. F. Franco, B. Varney, G. Sundaram, D. A. Brown, J. De Bie, C. K. Lim, G. J. Guillemin and B. J. Brew (2015). "Expression of the kynurenine pathway in human peripheral blood mononuclear cells: implications for inflammatory and neurodegenerative disease." PloS one**10**(6).
15. Khanra, R., S. Dewanjee, T. K. Dua, R. Sahu, M. Gangopadhyay, V. De Feo and M. Zia-Ul-Haq (2015). "Abroma augusta L.(Malvaceae) leaf extract attenuates diabetes induced nephropathy and cardiomyopathy via inhibition of oxidative stress and inflammatory response." Journal of translational medicine**13**(1): 6.
16. Mohlke, K. L., A. U. Jackson, L. J. Scott, E. C. Peck, Y. D. Suh, P. S. Chines, R. M. Watanabe, T. A. Buchanan, K. N. Conneely and M. R. Erdos (2005). "Mitochondrial polymorphisms and susceptibility to type 2 diabetes-related traits in Finns." Human genetics**118**(2): 245.
17. MP, G., A. Joshi, H. Joshi, D. Sathyanarayana, E. Subrahmanyam and K. Chandrashekhar (2010). "Antidiabetic potential of cow urine in streptozotocin-induced diabetic rats." 亚洲传统医药**6**(1): 8-13.
18. Nayak, S. S. and T. Pattabiraman (1981). "A new colorimetric method for the estimation of glycosylated hemoglobin." Clinica Chimica Acta**109**(3): 267-274.
19. Ohkawa, H., N. Ohishi and K. Yagi (1979). "Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction." Analytical biochemistry**95**(2): 351-358.
20. Palaian, S., A. K. Chhetri, M. Prabhu, M. Surulivelrajan and P. Ravi Shankar (2005). "Role of pharmacist in counseling diabetes patients." Journal of Pharmacology**4**(1).
21. Sheela, N., M. A. Jose, D. Sathyamurthy and B. N. Kumar (2013). "Effect of silymarin on streptozotocin-nicotinamide-induced type 2 diabetic nephropathy in rats." Iranian journal of kidney diseases**7**(2): 117.
22. Singh, B. M., M. A. Palma and M. Nattrass (1987). "Multiple aspects of insulin resistance: comparison of glucose and intermediary metabolite response to incremental insulin infusion in IDDM subjects of short and long duration." Diabetes**36**(6): 740-748.

23. Srinivasan, K., B. Viswanad, L. Asrat, C. Kaul and P. Ramarao (2005). "Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: a model for type 2 diabetes and pharmacological screening." Pharmacological research**52**(4): 313-320.
24. Tan, B. K. H., C. H. Tan and P. N. Pushparaj (2005). "Anti–diabetic activity of the semi–purified fractions of Averrhoa bilimbi in high fat diet fed–streptozotocin–induced diabetic rats." Life sciences**76**(24): 2827-2839.
25. Turko, I. V., S. Marcondes and F. Murad (2001). "Diabetes-associated nitration of tyrosine and inactivation of succinyl-CoA: 3-oxoacid CoA-transferase." American Journal of Physiology-Heart and Circulatory Physiology**281**(6): H2289-H2294.
26. Verma, P. K., V. K. Kamboj, M. K. Mishra and S. Ranjan "Antidiabetic Activity Of Ethyl Acetate Extract Of Pterocarpus Marsupium I Alloxa I Duced Diabetic Rats."
27. Vijayan, D. and G. Sibi "Pterocarpus Marsupium for the Treatment of Diabetes and Other Disorders."
28. Yadav, S., V. Vats, Y. Dhunnoo and J. Grover (2002). "Hypoglycemic and antihyperglycemic activity of Murraya koenigii leaves in diabetic rats." Journal of ethnopharmacology**82**(2-3): 111-116.