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Research Article

"Therapeutic Potential of Mitragyna Parvifolia Against Hyperglycemia, Inflammation, And Liver Apoptosis in Streptozotocin-Induced Diabetic Rats"

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Abstract

Background: Ethanolic extract of Mitragyna parvifolia leaves were used as a traditional use in the treatment of diabetic conditions. The aim of the current study was to find out the effect of *Mitragyna parvifolia* ethanolic leaves extract (MPE) on hyperglycemia developed by streptozotocin (STZ) induced type 2 diabetes in rats.

Materials and Method: An animal model of diabetes type 2 was developed by administration of STZ 90 mg/Kg to two-day old neonatal rat pups. Oral glucose test and parameters were examined of diabetic rat after the different dose of extracts. The rats were exist into following groups:

Group I –Normal control, Group II – STZ diabetic control, Group III –STZ Diabetic –orally administered MPEx at doses of 300 mg/kg, Group IV STZ-Diabetic – orally administered at doses of 500 mg/kg, Our study was confirmed that 8 weeks chronic treatment with MPE in a dose dependent manner (300 and 500 mg/kg) reduced hyperglycemia and significantly increased serum insulin, C-peptide. Histology of liver was performed after sacrificing the rats with euthanasia

Result: The ethanolic extract of MP was not show any acute toxicity up-to the dose of 2000 mg/kg and it was shown better glucose consumption in oral glucose tolerance test. Orally treatment of different doses of MP leaves extract decreased the level of serum glucose, reduce elevated levels of tumor necrosis factor α (TNF-α), interleukin IL-6 in the serum of diabetic rats.

After the study, we found that elevated blood glucose and lipids were bring back to near normal. In addition, MPE also reduce elevated levels of tumor necrosis factor α (TNF-α), interleukin IL-6 in the serum of diabetic rats.

Conclusion: We conclude that MPE exerted antidiabetic effects and reduced its associated complications such as dyslipidemia and inflammation. HE and confocal image of TUNEL assay showed that MPE reduced inflammation and apoptosis in diabetic rats.

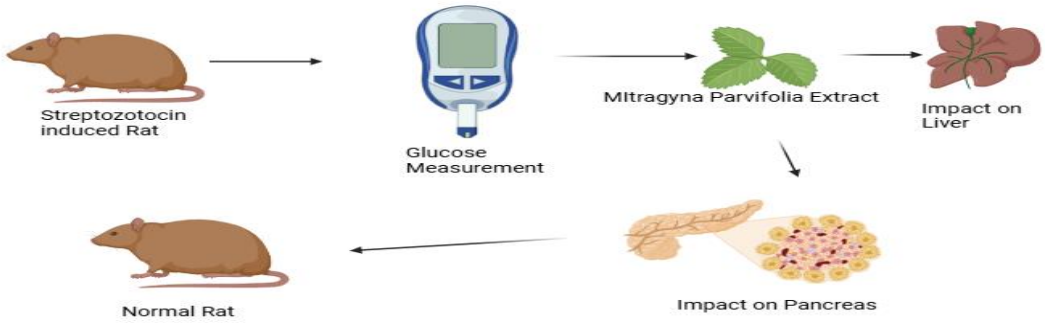


Fig: Graphical Abstract

Keywords: Insulin, Tumour Necrosis Factor-α, hepatotoxicity, cholesterol, triglyceride

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1. Introduction

Diabetes mellitus is a chronic metabolic disorder, characterized by hyperglycaemia and insufficient insulin secretion. The sedentary lifestyle, lack of exercise and unhealthy eating habits play a pivotal role in developing diabetes in people. In the global picture, India is the emerging hub of diabetes, and yearly, large numbers of diabetic patients are increasing in India, estimated to be around 212 million people (International Diabetes Federation, 2024). Oxidative stress plays an important role in the pathophysiology of diabetes and the related complications like cardiovascular disease, blindness, nerve damage and nephropathy (JennaLynn et al., 2012). Reactive oxygen species (ROS) is play main part in development of oxidative stress. An aerobic organism needs energy for proper biological functions, which serves as a prolonged fuel in cell mechanism. The ATP production in mitochondria requires an electron from the reduced substrate, which occurs in complex reaction in the electron transport chain (ETS) of mitochondria. The unused electrons from this process react and form free radicals (Egger and Swinburn, 2002). Thus, ROS leads to oxidation of important macromolecules including proteins, lipids, carbohydrates and DNA.

Diabetes mellitus is strongly linked with lipid disorders and plays a pivotal role in the development of cardiovascular diabetic complications. The major cholesterol concentration in plasma, triglyceride, LDL and VLDL abnormality found in type 2 diabetes patients. Diabetes type 2 rat groups were experimentally induced diabetes having the high level of plasma cholesterol (Balasee et al., 1972; Murali et al.; 2002).

Nowadays herbal drugs are getting a lot of attention, as they are a good source of alternative medicine in the diabetes treatment. The diabetes treatment with synthetic drugs is costly and associated with large no of side effects. And these treatments are for all over life required, where an herbal medicine in concept to treat patients by natural way to impact on disease. In addition, various newer anti-diabetic medicines like GLP-1 (glucagon like-peptide-1)-receptor agonist exenatide and the GLP-1-analogue liraglutide have caused side effects like nausea, headache, vomiting etc. Thus herbal medicines are gaining world-wide popularity these days. The herbal treatment effect is cure the body on natural way. Indian traditional medicine system has been using herbal drugs since ancient times. (Ohaeri et al, 2001). One of the ancient book AYURVEDA is having the lots of written herbal treatment for the various life style diseases.

Our laboratory first investigated the effect of MP leaves in diabetes (Shukla and Srinivasan, 2012). These leaves alkaloids were shown excellent effect against oxidative stress in diabetic rats (Shukla and Srinivasan, 2012). These leaves consists of various alkaloids- 16,17-dihydro-17b-hydroxy isomitraphylline and 16, 17-dihydro-17b-hydroxy mitraphylline together with two known alkaloids- isomitraphylline and mitraphylline, which was reported in 2006 (Pandey et.al,2006 Feillet-Coudray). These leaves possess anti-diabetic and anti-oxidative stress effect, which may be due to the presence of alkaloids, flavonoids, saponins, glycosides and terpenoids in the extract.

The aim of present study was to find out the protective effect of MP leaves extracts against hepatotoxicity, hyperglycaemia and proinflammatory (markers of oxidative stress) in STZ induced diabetes rat model.

2. Material and methods:

2.1 Chemicals

The chemicals and reagents were obtained from Lobachemie, India and SD fine chemicals, India; and streptozotocin was procured from Sigma, USA. All chemicals and reagents were used in the studies of analytical grade.

2. 2 Animals and diabetes induction:

Wistar albino rats either sex, weight 160 -250 gm, were procured from the animal house of Delhi Institute of Pharmaceutical Sciences and Research (DIPSAR), New Delhi. The approval was given by the Institutional Animal Ethics Committee of DIPSAR for the animal model development and experiment. The animals were maintained under the standard laboratory conditions at temperature 22 ± 3ºC, 12h: 12h light and dark cycles maintained during the study. The animals were housed in propylene cages and were fed on the standard rat pellets and water adlibitum according to the guideline of CPSCEA. To development of experimental model of Non-Insulin-Dependent Diabetes Mellitus (NIDDM), Streptozotocin (90 mg/kg) was dissolved in a freshly prepared citrate buffer (4.5 pH) and administered *i.p.* to a group of 2 days-old pups (Weir et al.1981). Another group received citrate buffer only. Both group pups were weaned for 30 days and after six weeks, fasting plasma glucose was checked. Those rats which had more than

180 mg/dl of blood glucose were considered diabetic. All the animal experiments were carried out under the CPCSEA guidelines.

2.3 Experimental Design:

The present study was performed in four groups, comprising six (n=6) animals in each group. MPE (300mg/kg and 500 mg/kg) was dissolved in carboxy methylcellulose (CMC) and administered rats orally.

Study was divided in a four groups

Group I- Control groups were administered vehicle orally (CMC, 0.5% w/v, 5 ml/kg)

Group II- Diabetic control rats were developed by STZ in a single dose (90 mg/kg, i.p. to two-day pups).

Group III- Experimental groups were orally administered MPEx in CMC solution (5 ml/kg) at doses of 300 mg/kg.

Group IV- Experimental groups were orally administered MPEx in CMC solution (5 ml/kg) at doses of 500 mg/kg.

The drug was administered on daily basis. At the end of the study, rats were anaesthetise and killed by cervical dislocation. The blood was withdrawn from the inferior vena cava for each rat and collected in heparin-coated tubes. The blood samples were centrifuged at 1000g for 15 min at 4ºC and the plasma was carefully separated from the serum. The plasma was used for the estimation of insulin, IL-6, C-RP, cholesterol and TNF-α. The livers were excised, rinsed in ice-cold saline and was stored at -20ºC for further studies.

2.4 Ethanolic extract preparation:

MP leaves were collected from the Pusa campus, New Delhi, India and plant leaves were identified by the Dr. H.B Singh NISCAIR (National Institute of Science Communication and Information Research), New Delhi. A voucher specimen was deposited in the NISCAIR, voucher no. Extract NISCAIR/RHMD/CONSULT/2008-09/1179/211.

These leaves washed with water three times, air dried, powdered and extracted with ethanol (90%) in a soxhlet apparatus for 50 hrs. The extract was evaporated to dryness under reduced pressure to give the solid residue. The residue was stored in a deep freezer until used.

2.5 Biochemical analysis

2.5.1 Fasting Plasma Glucose:

At the end of the experiment, blood-glucose concentration was estimated in the venous blood from the tail using accu-check (Roche Diagnostics Gmbh Mannheim, Germany) for the study of the chronic effect of MPE on diabetic rat's glucose level in plasma.

2.5.2 Oral Glucose Tolerance Test (OGTT):

Chronic effect of MPE administration was estimated on oral glucose tolerance of diabetic rats. OGTT was performed at the end of the study. After an overnight fast, the rats were given glucose orally (2g/kg body weight), and the blood-glucose levels were determined in tail blood samples 0, 30, 60 and 120 min. after the glucose administration.

2.5.3 Measurement of Plasma Insulin and biochemical parameter:

Plasma insulin (Crystal chem, USA), IL6 (Cusabiotech,USA), C-RP (Immunology Counsultants Laboratory,USA), Cholesterol (Biovision, USA), and Tumor Necrosis Factor-α (TNF-α) (Raybiotech, USA) were determined by rat enzyme-linked immunosorbent assay (ELISA) technique, as per instructions of the manufacturers provided in the ELISA kits. Triglyceride (Bioassay Systems, USA) was measured by the colorimetric assay.

2.5.4 Histopathological examination:

Livers were removed from the rats after anesthesia. They were washed with phosphate buffer, and these organs were stored in 10 % formalin solution. Tissues were processed by isopropyl alcohol. After clean with xylene and then fixed in paraffin wax. Consequently, wax blocks were prepared and then sections cut by the microtome, stained by hematoxylin and eosin method, and after that photographed.

2.5.5 Terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling (TUNEL ASSAY) and Confocal Laser Scaning Microscopy

Terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling (TUNEL) staining was carried out with an apoptosis detection kit (Biovision, USA) on the 5µ paraffin-embedded tissue sections, according to the manufacturer's protocol. Apo-BrdU stained (TUNEL stained) were analyzed with an Olympus Fluoview 1000 confocal microscope (Olympus, Tokyo), FV10- ASW software (version 1.7; Olympus) and oil uplan filter 60 objectives. Confocal microscopy was done in Advance Instrument Research Facility of Jawahar Lal Nehru University, New Delhi (Negoescu et al., 1998).

2.6 Statistical Analysis

Statistical analysis was done on three groups: control, diabetic and MPE treated experimentally induced diabetic group. Comparisons between different groups were performed by using one-way analysis of variance (ANOVA) followed by a TUKEY Multiple Comparison Test. The diabetic group was compared with the control and MPE treated group. Data was represented as mean and SEM for each group of rats. P-values <0.05 (sigma plot 11) were considered statistically significant.

3. Result:

3.1. Effect on Hyperglycaemia and Oral Glucose Tolerance Test:

Table 1 shows the effect of MPE administration in diabetic rats- plasma glucose level was significantly reduced in comparison to the diabetic group (P<0.005). Plasma glucose level was markedly augmented in the experimental diabetic group (P<0.005) in comparison to the control rats group (Fig.1A).

Control, diabetic and MPE administered rat groups were shown a significant changes in the blood-glucose levels after the single dose glucose (2gm/kg). Blood glucose level in diabetic rat group was significantly elevated after the glucose administration (45 min with respect to

control) and throughout the period of experiment. In contrast, M.P treated diabetic rat groups, blood-glucose level was lowered, and normal glucose level (near to control) was obtained; although after 45 min. of glucose administration, it reached the peak level (Fig 1B).

3.2 Effect of M. Parvifolia on Insulin:

As shown in Fig. 2, plasma insulin in the diabetic rats showed two-fold rise in comparison to the normal rats. MPE administered diabetic rats showed a marked decrease in plasma insulin level as compared to the untreated diabetic rat group ($P<0.005$).

3.3 Effect of M. Parvifolia on plasma lipid:

The data of Serum levels of lipids, Total Cholesterol (TC), triglyceride (TG), High-Density Lipoprotein-cholesterol (HDL-C) and atherogenic index in normal and experimental animals in each group is shown in Table 2. In STZ administered rat group, serum cholesterol and triglyceride levels were significantly augmented ($P<0.001$). On the other hand, MPE extracts marked lowered the serum level of TC and TG in a dose-dependent manner when it was compared with the diabetic group. Serum HDL-C good cholesterol level was significantly decreased in diabetic rat as compared to the control rat. Accordingly, MPE treated rat group, serum HDL-C level was significantly augmented ($P<0.001$).

3.4 Effect of M. Parvifolia on proinflamatry cytokines:

As far as proinflammatory cytokines concerned in diabetic experimental model development, treatment with STZ (Fig.2) significantly increased the serum levels of C-RP and IL6. Eight weeks of chronic treatment with MPE significantly diminished STZ-elevated C-RP and IL-6 level ($P<0.05$) in plasma. Plasma TNF-α levels showed significant was increased in the STZ administered group as compared to the control group (Fig. 2). The chronic MPE administered group showed significant reduction in Plasma TNF-**α** level as compared to the diabetic group.

3.5 Effect on M. Parvifolia Liver tissue:

The control group animals showed a normal architecture, hepatocytes separated by the sinusoids as shown in Fig 3 A. Bile duct and portal vein was located in the portal area. The hepatocytes were centrally nucleated, stained and had clear nucleolus with polygonal shape. In STZ administered group, moderate portal vein inflammation occurred and number of binucleated cells increased (Fig 3B). On the other hand, chronic MP extract administered group showed significant decrease in portal vein inflammation and number of binucleated cells (Fig 3C).Image J software analysis of liver image shows a significant effect of treatment on the inflammation in diabetic rats(Fig.3G).

3.5 MP effect on apoptosis

We examined the effect of MP on liver apoptosis in STZ diabetic rats after eight-week treatment. As per result, STZ administered rat's shows DNA fragmentation in liver. TUNEL method is the safest way to measure apoptotic DNA fragmentation in STZ administered rat (Negoescu et al., 1998). STZ induced rats shows liver fibrosis, kuffer cells (KC's) engulfing apoptotic cells of various types including hepatocytes, neutrophills and erythrocytes. TUNEL assay was performed in order to characterize the effect of diabetic state on induced apoptosis in the liver. Here by using laser confocal scanning microscopy, we initiate that KC's could undergo apoptosis in STZ induced liver fibrosis. Apoptosis in diabetic group was high when compared with normal. After the 8 weeks of chronic MP administration, significantly decreased apoptosis (Fig.4).

Discussion:

The common name of *Mitragyna parvifolia* (Roxb.) is Kaim. The tree is present throughout India in deciduous and evergreen forests. The traditional use of MP is as a local folkare medicine to treat fever, colic & muscular pain (Shellard et al. 1974, 1971). Diabetes is a group of metabolic disorders and is associated with augmented levels of plasma glucose. The persistent increase in plasma glucose level increases the risk of diabetic complications. Streptozotocin-induced diabetes is a well-established model for diabetes studies (Weir et al., 1981) and is used for the screening of drugs (vorra et al. 1989).

STZ induced diabetic model is characterized by increased glucose and insulin levels as compared to the control group. As we know, insulin controls the blood-glucose level and acts on the liver, muscle, and target tissues (Gayathri and Kannabiran, 2008). Insulin deficiency causes activation of hormone-sensitive lipase (HSL) accompanied by enhancement of the release of free fatty acids (FFA) from the adipose tissue (Al-Shamaony, et.al, 1994). Chronic MP administration resulted in reduced plasma levels of glucose and insulin as compared to the normal diabetic group. Insulin-controlled blood-glucose level and oral glucose tolerance test were carried out after the eight weeks of treatment. Chronic treatment with MP extracts (300 and 500 mg/kg) effectively controlled the hyperglycaemia and maintained normal fasting glucose in STZ induced rats; it also decrease glucose excursion in diabetic rats as indicated by its effect on OGTT. Lowering of glucose exursion in diabetic rat due to DPP-IV inhibition by its alklaloids (Shukla and Srinivasan, 2012). Long-term administration of DPP-IV inhibitors improves the peripheral and hepatic insulin sensitivity, and levels of active GLP-1 and Glucose-dependent insulinotropic polypeptide (GIP). In addition, glucose tolerance, β-cell glucose responsiveness and reduced hyperinsulinemia were augmented in VDF rat.

CRP, a pentameric protein produced by the liver, is a well known pro inflammatory marker. Hyperglycemia and hyperinsulinemia are associated factor the increase of serum CRP levels non-controlled type II diabetic subjects (Martha and Fernando, 1999). Several studies demonstrate that CRP remained a significant predictor of diabetes risk, even after adjusting with body mass index, family history of diabetes mellitus, smoking and

other factors (Pradhan et al. 2005). TNF-α, and IL-6-proinflammatory cytokines and acute-phase reactants are involved in multiple metabolic pathways relevant to insulin resistance, including insulin regulation, reactive oxygen species, and lipoprotein lipase action (Crook M.et al. 2004).

In STZ administered rats, serum levels of TNF-α, C-RP and IL-6 were increased. Evidence suggests that the body inflammation is a key regulator in adipose tissue, in particular visceral adiposity. Various studies suggest that inflammatory mediators pose a novel risk of atherosclerosis and diabetic complications (Pickup, et.al.1999; Ross R, et.al.1999). Proinflammatory cytokines were significantly diminished in diabetic treated rats. Adipose tissue released inflammatory cytokines (IL6 and TNF-α) have shown the effect on Insulin Resistance (Hotamisligil,et.al,1993; Orban,et.al,1999). TNF-α and IL-6 stimulates acute phase protein in liver. CRP is primary synthesized in the liver and controlled by the pro-inflammatory cytokines IL-6 and tumor necrosis factor-alpha (TNF-α) (Gabay,et.al,1999).

In the present study, we observed TNF-α, C-RP and IL-6 concentrations were significantly decreased in MPEx groups in comparison with the diabetic group ($p < 0.001$).

Diabetes mellitus characterized by hypertriglyceridemia and hypercholesterolemia (Khan et al., 1995; Mitra et al., 1995). In diabetes, insulin resistance accompanied with dyslipidemia which characterized by TC, TG, LDL and HDL. In this LDL increase and HDL decrease when diabetic is confirmed. In the present study, we found that the hypertriglyceridemia with low HDL cholesterol and moderately elevated serum total and LDL cholesterol is the typical lipoprotein profile in a diabetic rat (DeFronzo and Ferrannini 1991, American Diabetes Association 1993).

There so many evidences suggest that insulin control cholesterol synthesis in liver and play important role in lipid metabolism (Luc G, et. al, 1991). Steinberger J, suggest that hyperinsulinemia enhance hepatic VLDL synthesis and, thus, may directly contribute to the increased plasma TG and LDL cholesterol levels observed in obese adolescents.

The antilipolytic effect of insulin is reduced in adipose tissue leading to increased release of fatty acids (Reaven 1988). Liver is exposed to a large free fatty acid load, which could induce hepatic insulin resistance (Carey et al. 1996) and provide substrates for increased VLDL production (Björntorp 1991). As a matter of fact, abnormal VLDL production and deranged activity of lipoprotein lipase have been linked to insulin resistance (Pollare et al. 1991, Malmström et al. 1997).

Increase TC and LDL cholesterol major risk factor for cardiovascular disease, whereas increased HDL cholesterol is associated with a decrease in cardiovascular disease risk. It is well known that HDL plays an important role in the transport of cholesterol from the peripheral blood to the liver by the reverse cholesterol transport pathway.

Significant lowering of TC and rising of HDL cholesterol are very desirable biochemical states. MPEx has the potential to reduce TC and LDL and increase HDL in comparison to the diabetic rats.

The marked increase in serum triglycerides, total cholesterol, LDL-cholesterol and decreased HDL cholesterol observed in diabetic rats is in agreement with the findings of Nikkila and Kekki 1973. The most usual lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia (Khan et al., 1995; Mitra et al., 1995). The abnormal high concentration of serum lipids in diabetes type 2 is due to lipase; insulin activation of lipase in adipocytes are decreased which augments the movement of free fatty acids from the fat depots. Thus, STZ produce excess FFA in plasma after diabetes development and promote their conversion into phospholipids and cholesterol in liver. Both substances along with triglycerides are released from the liver (Bopanna,et.al,1979). However, treatment with the MP extracts normalized the plasma lipid status, which was presumably mediated by the control of lipid metabolism.

Our objective of the study was to investigate the hepatoprotective effect of MPE in diabetes type 2 rats. In STZ induced diabetic rats, liver showed moderate inflammation and no of binucleated cells was increased. When we examined the tissue image with image J software(USA), we found percent cell area of inflamed cells was increased in diabetic rats slide in normal. After chronic administration of MPE, treated slide shows decreased inflamed cell area and number of binucleated cells in compar diabetic rat liver slide image(Fig.3G).

Our previous findings demonstrate that MPEx treatment impairs the increase of $^{\bullet}OH$ production generated by the diabetic state in the rat liver. In diabetic rat, high levels of this reactive species play an important role in the increase of hepatic LPO. The balance between the expression of pro-apoptotic and anti apoptotic proteins is disturbed after the increase of hepatic LPO (Shukla and Srinivasan, 2012).

Confocal laser scaning microscopy results of TUNEL assay demonstrate apoptosis occurs in the diabetic liver. STZ induced diabetes, $OH^{\bullet}$ contributes partially to be mitochondrial c release and caspase 3 activation, which are associated with hyperglycemia induced liver apoptosis. Figure 4 clearly shows, DNA fragmentation was decreased as compared with the diabetic section. Figure 4E grey image of the diabetic control shows more number of whiter bit radiance in circle but after MP administration, number of white bit radiance decreased significantly (Fig.4H). The present results demonstrate that MP reduces apoptosis in STZ induced liver.

In summary, present experiment imparts novel information on the hyperglycemia which can be prevented by an MPEx possibly by suppression of the action of proinflammatory cytokines. Hepatoprotective activity of MPEx in STZ induced diabetes type 2 rats are due to the reduction of oxidants in the hepatic tissue. MPEx produces strong action on hyperglycaemia, proinflammatary cytokines, hypertriglyceridemia and hypercholesterolemia as well as augments the hepatic antioxidant defense system in STZ-induced rats. Histopathological and laser confoal scaning microcopy of TUNEL slides studies also support this view. Our

findings strongly suggest that MP extract work as antidiabetic in STZ-induced hepatotoxicity in rats.

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Availability of data and materials: The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Authors' contributions

The authors' contributions were as follows: Ashutosh: designed the research (project conception, development of overall research plan, and study oversight, conducted the research (hands-on conduct of the experiments and data collection); others analyzed the data or performed the statistical analysis; Ashutosh had primary responsibility for the final content. All others had no conflict of interest. All authors read and approved the final manuscript.

Competing interests: The authors declare that they have no competing interests.

Consent for publication: Not applicable.

Ethics approval and consent to participate: The study was approved by the Institutional Animal Ethics Committee of DIPSAR for animal model development and experiment.

Abbreviations

M.P: *Mitrgayna Parvifolia*
MPE: *Mitrgayna Parvifolia Extract*
STZ: streptozotocin
TNF-α), tumor necrosis factor α
IL-6 : Interleukin
TUNEL assay: terminal deoxynucleotidyl transferase dUTP nick end labeling
HE: Heamatoxylin
ROS: Reactive oxygen species
ETS: Electron transport chain
DNA: DeoxyRibo Nucleic Acid
LDL: Low Density Lipoprotein
VLDL: Very Low-Density Low protein
GLP-1: Glucagon like-peptide-1
DHIM: 16,17-dihydro-17b-hydroxy isomitraphylline
CMC: Carboxy Methyl Cellulose (CMC)

Author's contribution

Ashutosh Shukla carried out research work like extraction, animal model development, and immunoassays. Other authors conceived of the study and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript

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Graphical Abstract

Diabetes induced by 90 mg/kg in two days pups of rats. Eight weeks of administration of *Mitragyna parvifolia* (MP) on diabetic rats showed significant effect on Liver, TNF-α and Plasma insulin was markedly reduced in diabetic rats. Hepatotoxicity developed by STZ, was significantaly reduced.

Figure Captions

Fig.1 A effect of MP on fasting plasma glucose in a dose dependent manner

Fig.1 B effect of MPE on oral glucose tolerance test (OGTT)

[Mean ± S.E.M. One way ANOVA followed by Tukey's Multiple Comparison Test. + P<0.05, ++ P<0.01, ¥ P<0.001 compared with Diabetic control & Diabetic treated group.*P<0.05, $ P<0.01, # P<0.001 compared control with Diabetic control]

Fig.2 Effect of Miragyna *parvifolia* extract (MPEx) on Insulin and proinflammatory cytokines in serum of STZ-

treated rat eight weeks chronic oral dose administration of 300 and500 mg/kg b.w. D=diabetic ; mean ±SE; n = 6.

Fig.3 Photograph of rat liver shows (HE, × 40). A: Liver of a control rat showing normal hepatocytes and normal architecture; B & E Liver section from a STZ administered rat demonstrating the destruction of architectural pattern, moderate inflammation of portal area(B & E image shows moderate inflammation in yellow and red line circle with arrow) C: Liver section from a MP treated rat showing normal lobular architecture no necrosis or fatty changes or any inflammatory reaction can be seen. D, E, F is the image of A, B, C which is created by the Image J software in this clearly showing the inflammation in STZ administered (E), after treatment, inflammation were reduced (F).

Fig. 4 Effect of MP on STZ Diabetic rat. Sections were obtained with Terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling (TUNEL ASSAY). Tunel assay distinguishes the apoptosis in STZ diabetic rat. Confocal analysis of tunel section A, B, C Control rat, D, E, F STZ Diabetic rat and G, H, I MP traeted. B, E, H is the grey image of the A, D, G. C, F, I is the merge image of A, D, G and B, E, H. Kupffer positive cells in a green. White bit radiance in grey image (B, E, H) and merge (C, F, I) represent apoptosis.

Table Captions

Table 1- Levels of plasma glucose and insulin of control, and diabetic rats after 8 weeks of treatment with MP

Table 2 Levels of Total cholesterol (TC), HDL and TG of control and diabetic rats after 8 weeks of treatment with MP

Table 1- Levels of plasma glucose and insulin of control, and diabetic rats after 8 weeks of treatment with MP

Groups	FPG	Insulin
control	89.333 ± 2.67	1.185 ± 0.156
Diabetic	197.66 ± 5.33	2.335 ± .144
D + M.PEx(300mg/kg)	125.167 ± 8.19	1.351 ± .121
D + M.PEx(500mg/kg)	112.167 ±3.86	1.255 ± 0.084

Table 2 Levels of Total cholesterol (TC), HDL and TG of control and diabetic rats after 8 weeks of treatment with MP

Groups	TC	HDL	TG
control	112 ± 14.14	48.41062 ± 6.02	84.28 ± 5.31
Diabetic	262 ± 16.04	22.91698 ± 2.635	160.61 ± 2.16
D + M.PEx(300mg/kg)	138 ± 17.48	42.7979 ± 5.07	122.5 ± 10.81
D + M.PEx(500mg/kg)	126.5 ± 13.79	44.76905 ± 5.71	93.62 ± 2.19

Figure:

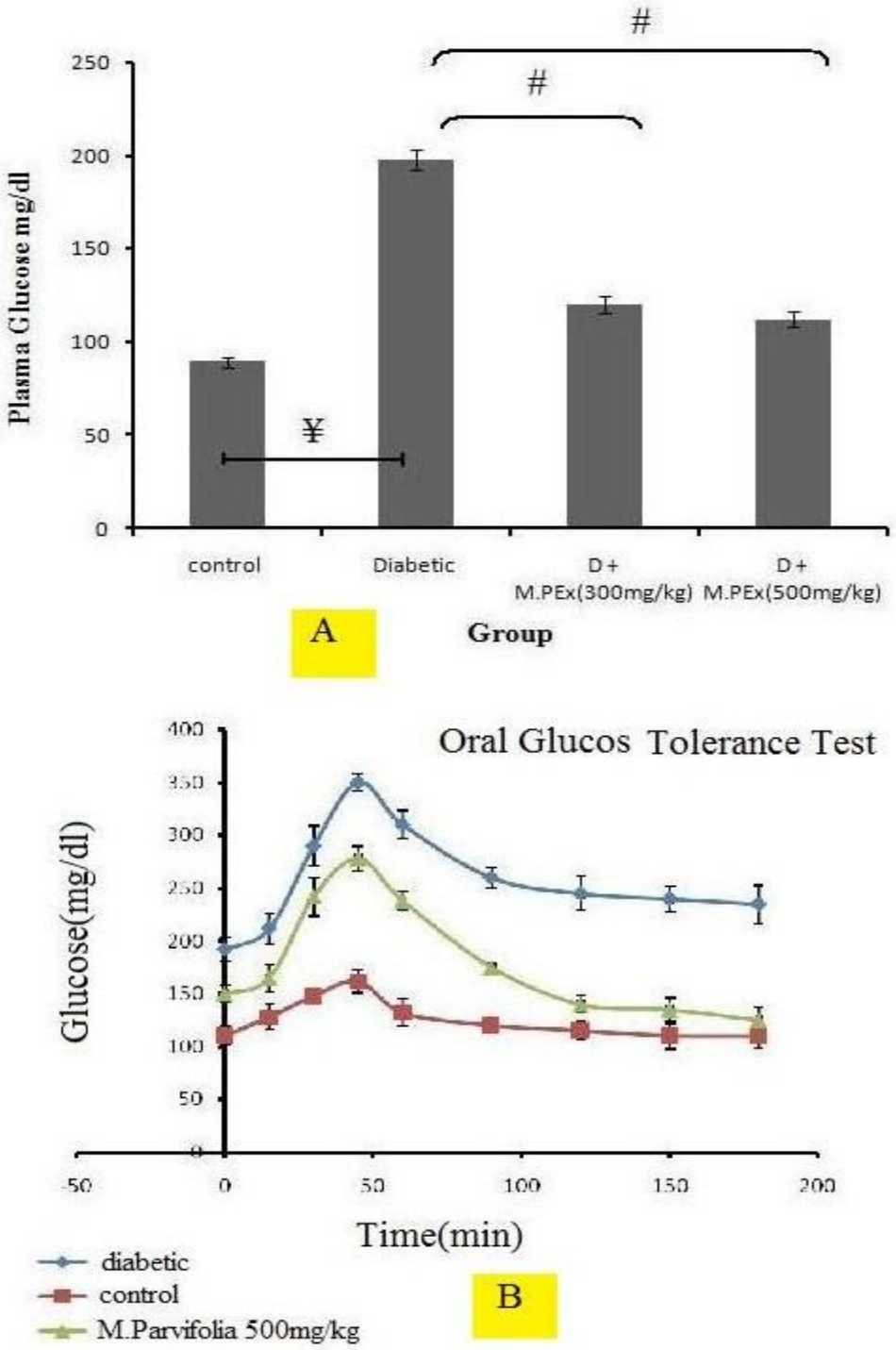

#
#
250
200
150
100
50
0
Plasma Glucose mg/dl
¥
control
Diabetic
D+ M.PEx(300mg/kg)
D+ M.PEx(500mg/kg)
A
Group
Oral Glucos Tolerance Test
400
350
300
250
200
150
100
50
0
Glucose(mg/dl)
-50
0
50
100
150
200
Time(min)
diabetic
control
M.Parvifolia 500mg/kg
B

Fig.1

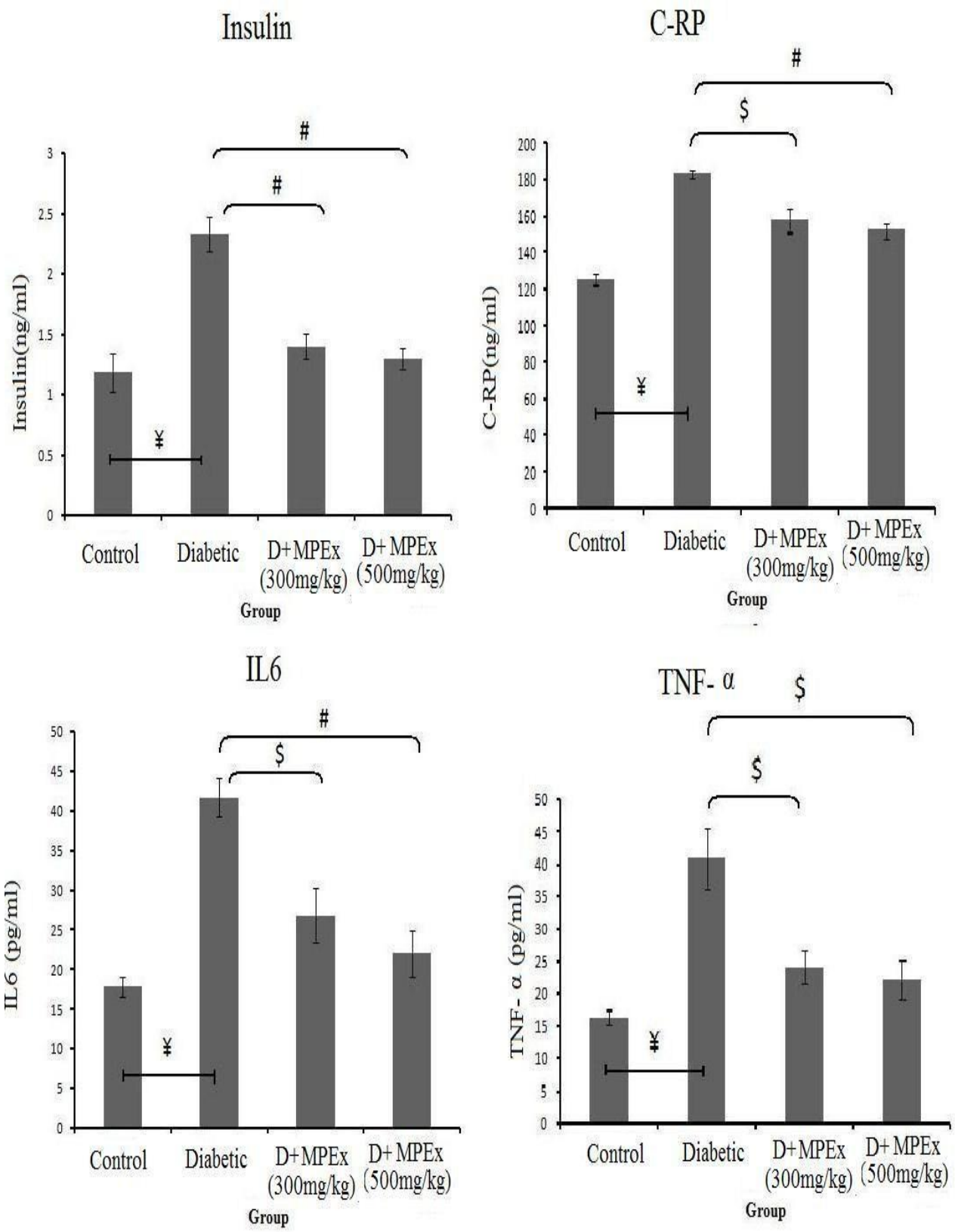

Insulin
Insulin(ng/ml)
Control
Diabetic
D+MPEx (300mg/kg)
D+MPEx (500mg/kg)
Group
C-RP
C-RP(ng/ml)
IL6
IL6 (pg/ml)
TNF- α
TNF- α (pg/ml)

Fig 2

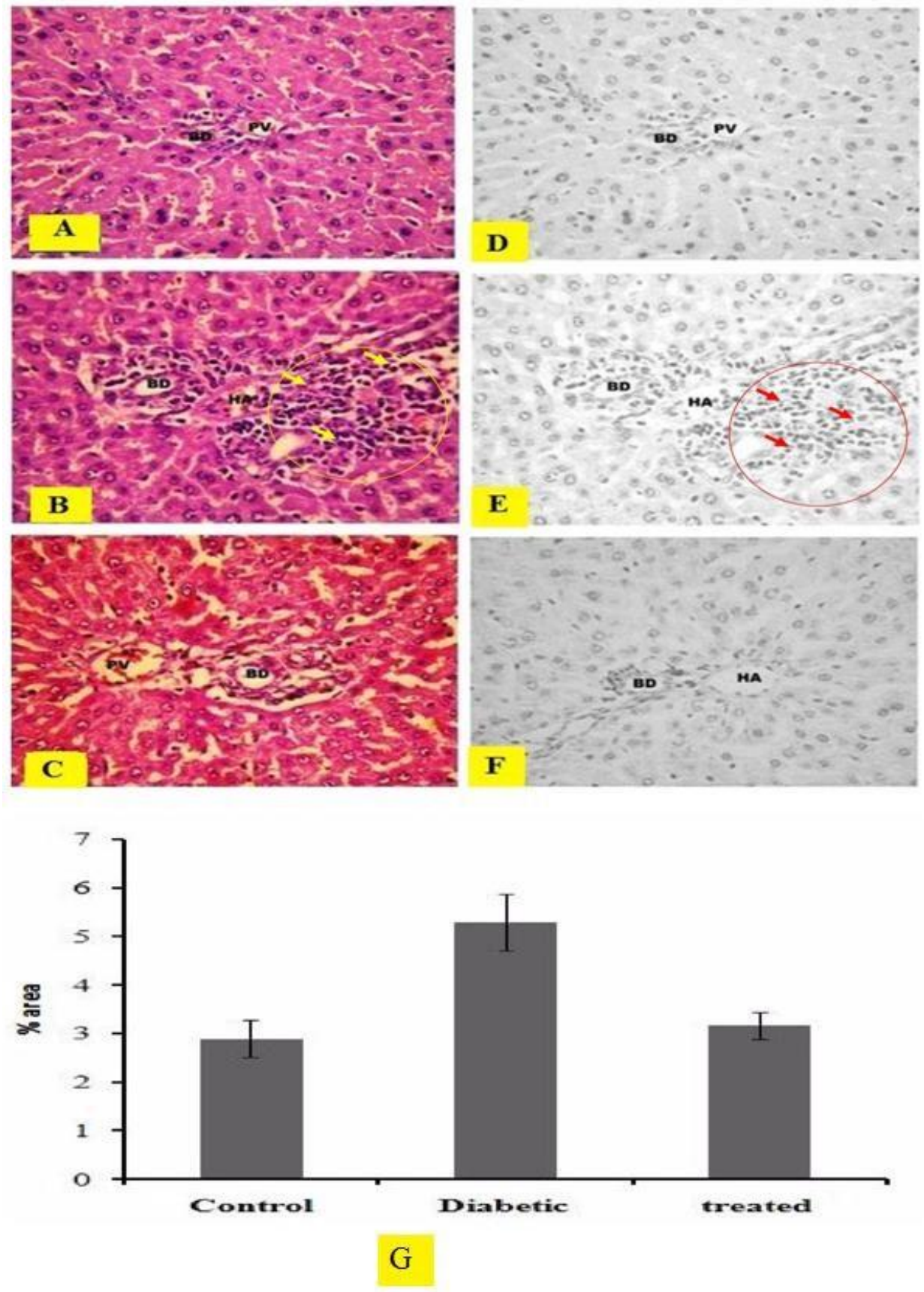

A
BD
PV
D
BD
PV
B
BD
HA
E
BD
HA
C
PV
BD
F
BD
HA
7
6
5
4
3
2
1
0
% area
Control
Diabetic
treated
G

Fig 3

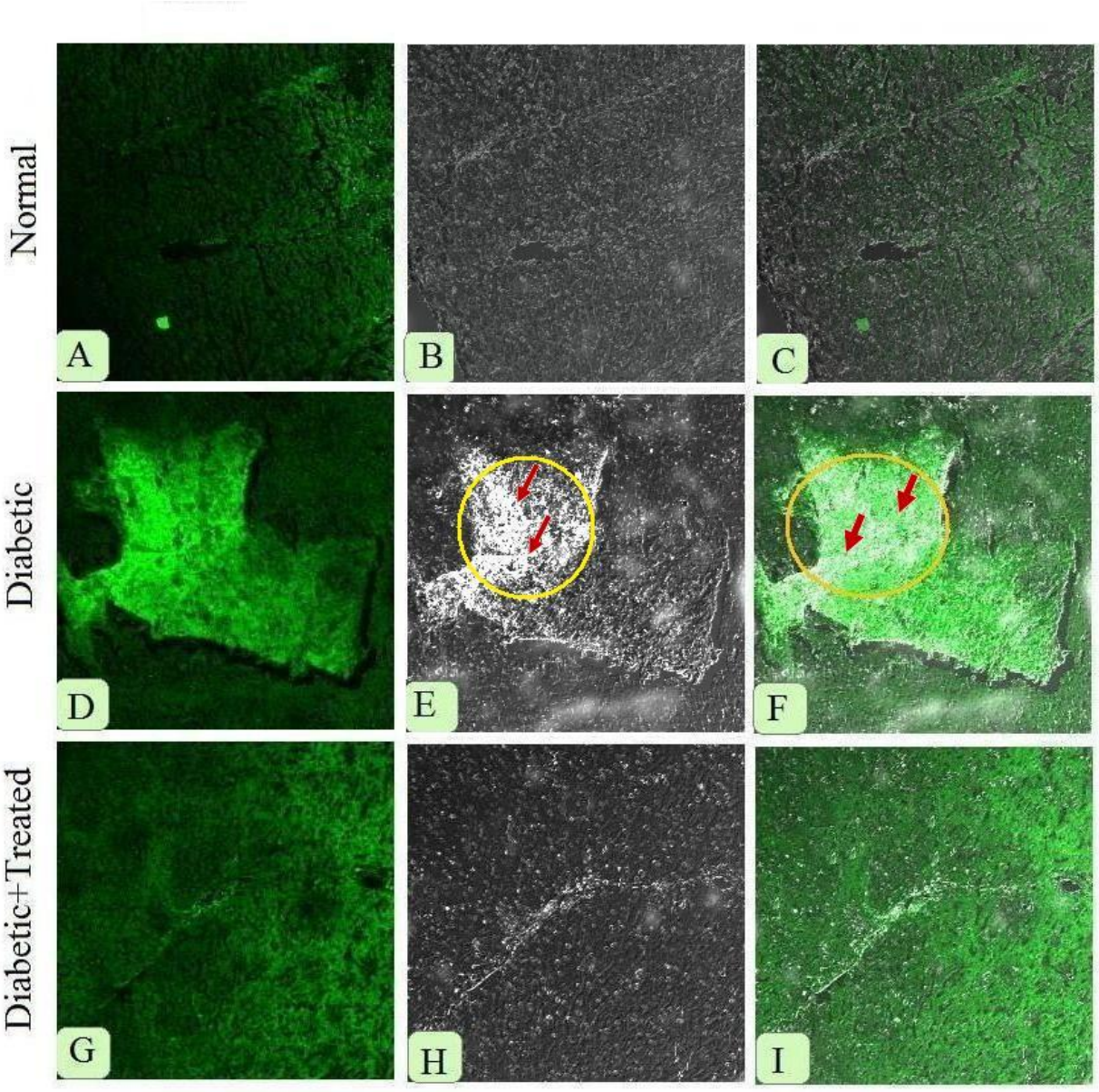


Fig. 4

Bullet point

1. M.Parvifolia leaves extract reduced blood glucose levels in diabetic rats.
2. M.Parvifolia leaves extract reduced proinflammatory markers in diabetic rats.
3. M.Parvifolia leaves extract reduced apoptosis in diabetic rat liver.