Research Article

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The effect of Madhumeha Kusumakar Rasa – an *Ayurved* medicine – in insulin resistance

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Abstract

Objectives: Madhumeha Kusumakar Rasa (MKR) is an Ayurved formulation having a strong pharmacological base for diabetes management. This study aimed to validate MKR's efficacy in dexamethasone-induced insulin resistance (IR).

Methods: Albino Wistar rats were divided into four groups. Group 1 served as the normal control, Group 2 received dexamethasone 1.5 mg/kg (i.p.), Group 3 received dexamethasone and metformin 200 mg/kg (p.o.), and Group 4 received dexamethasone and MKR 236 mg/kg (p.o.). Animals were evaluated for serum glucose levels and glucose tolerance, serum insulin, Homeostatic model assessment of insulin resistance (HOMA-IR), Homeostatic model assessment of insulin sensitivity (HOMA-IS), fasting glucose to insulin ratio (FGIR), and lipid parameters. Pancreas, liver, and kidneys were evaluated for reduced Glutathione (GSH) and Malondialdehyde (MDA) levels. These tissues were also evaluated for histopathological changes.

Results: MKR showed significant improvement in serum glucose and glucose tolerance, serum insulin and HOMA-IR, HOMA-IS, and FGIR. It also showed a significant improvement in lipid parameters as compared to the dexamethasone-treated group. It prevented depletion of GSH levels and elevation in MDA levels. These effects were supported by histopathological analysis.

Conclusions: MKR treatment significantly attenuated dexamethasone-induced IR. This study validates the mechanism of the anti-diabetic potential of MKR.

Keywords: ayurved; ethnopharmacology; insulin resistance; phytomedicine; type 2 diabetes mellitus.

Introduction

Diabetes is one of the most frequently encountered metabolic disorders in today's world. The World Health Organization has labelled diabetes as a serious health condition. The International Diabetes Federation estimated that 463 million adults lived with diabetes worldwide in 2019. This figure is estimated to increase to 578 million by 2030 and reach 700 million by 2045 [1, 2].

Type 2 diabetes mellitus (T2DM) is characterized by insulin resistance (IR), hyperglycemia, and hyperinsulinemia caused due to impaired insulin signaling. T2DM accounts for nearly 90–95% of the total cases of diabetes and is associated with other metabolic disorders like obesity and cardiovascular disorders (CVD) [3–5]. Typically, refractoriness of insulin receptors to the action of insulin causes hyperglycemia in IR. Additionally, pancreas is not able to compensate with the increased peripheral demand of insulin because of IR leading to β-cell degeneration in advanced T2DM [5]. In T2DM IR contributes to increased hepatic glucose production and decreased glucose uptake in adipose and muscle tissues [6].

Conventional therapy for T2DM is associated with many adverse effects making it imperative to look for newer therapeutic strategies for the management of T2DM and its associated conditions. Madhumeha Kusumakar Rasa (MKR) is an *Ayurved* formulation prescribed for diabetes management. The ingredients of MKR have a synergistic action and work in unison to tackle T2DM. Additionally, all the

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ingredients of MKR (Table 1) are individually documented for their anti-diabetic effect.

This study aimed to evaluate the effect of MKR in dexamethasone-induced IR in albino Wistar rats. Dexamethasone is known to induce T2DM by down regulating insulin receptor substrate-1 and directly impairing GLUT-4 translocation [4]. In the present study, the mechanism of the anti-diabetic activity of MKR was estimated by evaluating serum glucose and insulin, serum cholesterol, low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), high-density lipoprotein (HDL), and triglycerides. IR being the pathological hallmark of T2DM, the extent of IR was evaluated by estimating insulin indices like Homeostasis model assessment of insulin resistance (HOMA-IR), Homeostasis model assessment of insulin sensitivity (HOMA-IS), and fasting glucose to insulin ratio (FGIR). The extent of glucose tolerance was measured by oral glucose tolerance test (OGTT). Malondialdehyde (MDA) and reduced glutathione (GSH) levels were estimated to evaluate the effect of MKR on dexamethasone-induced oxidative stress. Finally, histopathological study was performed to confirm the cytoprotective effect of MKR. All the results of MKR were compared with metformin, which is a standard drug used conventionally to manage IR associated with T2DM.

Materials and methods

Chemicals

MKR was obtained from Shree Dhootapapeshwar Limited, India, dexamethasone sodium phosphate injection (8 mg/2 mL vial) from Wockhardt Limited, India and Metformin hydrochloride (500 mg) from Cipla Limited, India. Insulin kit was obtained from Krishgen Biosystems, India and glucose, HDL, TG, and cholesterol kits from Erba Mannheim, India.

Table 1: List of active ingredients of MKR.

Ingredients	Quantity/ Tablet
Vasant Kusumakar Rasa (with Processed Gold)	10 mg
Shuddha Shilajatu (Processed Asphalt)	40 mg
Yashada Bhasma (Processed Zinc)	05 mg
Extract of Mamajjaka (*Enicostemma littorale*) whole plant	300 mg
Haridra (*Curcuma longa*) rhizome choorna	50 mg
Amalaki (*Emblica officinalis*) Fruit pericarp choorna	50 mg
Guduchi (*Tinospora cordifolia*) stem choorna	50 mg
Processed in decoctions of Bilva (*Aegle marmelos*) leaf and Asana (*Pterocarpus marsupium*) heartwood	q.s.

Animals

24 male albino Wistar rats (200–250 g) were procured from Bharat Serum and Vaccines Pvt. Ltd. and housed in clean propylene cages containing husk bedding under standard conditions of temperature (24 °C ± 2 °C), relative humidity (50% ± 5%) and light (12 h light/dark cycle). The animals were provided standard pellet diet and water as per the study protocol and acclimatized for seven days prior to the start of the study. The study protocol was approved by the Institutional Animal Ethics Committee (Protocol number- KMKCP/IAEC/181903).

Experimental design

After acclimatization, rats were randomly divided into four groups of six animals each. The day when any of the experimental drugs were administered was marked as day 1 and all the study drugs were administered for 28 days. Dosing was as follows: Group I (Normal Control) - distilled water *p.o.*, Group II (Toxicant) - dexamethasone 1.5 mg/kg/day *i.p.*, Group III (STD) - metformin 200 mg/kg/day *p.o.* + dexamethasone 1.5 mg/kg/day *i.p.*, Group IV (MKR) - dexamethasone 1.5 mg/kg/day *i.p.* + MKR 236 mg/kg/day *p.o.* MKR's dose was derived based on its human therapeutic dose (2,620 mg/day). Metformin and dexamethasone doses were selected based on previous literatures [7, 8]. Blood was withdrawn from the retro-orbital plexus under mild anesthesia and centrifuged at 2000 rpm for 10 min at –4 °C to obtain clear serum.

After 28 days, animals were euthanized by CO_2 asphyxiation. Pancreas, liver, and kidneys were used for histopathological studies and pancreatic tissues from each group were evaluated for antioxidant assays.

Biochemical estimation

Estimation of fasting serum glucose

Serum glucose was estimated using diagnostic kits on 0th, 7th, 14th, 21st, and 28th day according to the manufacturer's protocol (Erba Mannheim, India).

Estimation of fasting serum insulin

Serum insulin was estimated using a diagnostic kit on the 28th day according to the manufacturer's protocol (Krishgen Biosystems, India).

Based on the results of fasting serum glucose and insulin levels, the following insulin indices were estimated on day 28 based on previous literature [9].

- (HOMA-IR) = [Fasting glucose × Fasting insulin]/405
- (HOMA-IS) = 10,000/[Fasting glucose × Fasting insulin]
- (FGIR) = [Fasting glucose/Fasting insulin].

Estimation of serum lipid parameters

Serum cholesterol, triglyceride (TG) and HDL were estimated using a diagnostic kit on the 28th day as per the manufacturer's protocol (Erba

Mannheim, India). Other lipid parameters like LDL and VLDL were estimated based on previous literature [10]

LDL = Total cholesterol–HDL–TG/5

VLDL = TG/5

Estimation of OGTT

On the 28th day, after overnight fasting, all the animals were fed 2 g/kg of glucose. Serum glucose was measured at the 0^{th} min under fasting conditions and 30th, 60th, 120th, and 240th min after glucose load to assess the effect of MKR treatment on blood glucose levels in the glucose-loaded animals based on a previously published data [11].

Estimation of serum MDA levels

MDA was estimated as an oxidative stress marker based on previous literature [12]. Briefly, the pancreatic tissue was grinded and extracted with ethanol (80%) to make the tissue homogenate. The tissue homogenate (0.1 ml) was treated with 2 ml of TCA-TBA-HCl reagent (1:1:1 ratio) [15% trichloroacetic acid (TCA): 0.37 % Thiobarbituric acid (TBA): 0.25N HCl]. This mixture was placed in a water bath for 15 min. After cooling, the homogenate was centrifuged, and absorbance of the clear supernatant was measured at 535 nm.

Estimation of serum GSH levels

GSH was estimated using pancreatic homogenate based on a previous study [13]. Briefly, the pancreatic tissue was grinded in mortar and extracted with ethanol (80%) to make the tissue homogenate. 39.6 mg of 5,5′-dithiobis-(2 nitro benzoic acid) reagent was mixed in 10 ml phosphate buffer (pH-7.0). This reagent (0.02 ml) was added to 3 ml mixture of tissue homogenate, phosphate buffer (0.1 µmol, pH-8.0) and water (5 mL). This solution was then added in a photometer cell after color development in 2 min and absorbance at 412 nm was recorded. The formula used for the determination of GSH levels was [C = (A/e) × D] where, C is the unknown concentration, A is the absorbance at 412 nm, e is extinction coefficient (13,600/M/cm) and D is the dilution factor (10/3*3.02/3).

Histopathological evaluation

Pancreas, liver, and kidneys were fixed with neutral formalin (10%) and embedded in paraffin. The tissues were manually sectioned with a microtome to obtain 4-5 µm-thick paraffin sections. Dewaxed sections were then stained with Hematoxylin and Eosin (H&E) and observed under 100× magnification with Magnus MLX+ (Columbus).

Statistical analysis

GraphPad Prism Version 5, USA was used for the statistical analysis. The data is presented as Mean ± SD and evaluated by one-way ANOVA followed by post hoc Bonferroni test to detect inter-group differences. These differences were considered statistically significant if $p<0.05$, $p<0.01$, and $p<0.001$. To carry out OGTT assay, two-way ANOVA followed by post hoc Bonferroni test was performed to detect inter-group differences.

Results

Serum glucose and insulin levels and their indices

Dexamethasone treatment significantly elevated serum glucose and insulin levels than the normal control ($p<0.001$). HOMA-IR was significantly increased than the normal control ($p<0.001$), whereas HOMA-IS and FGIR were significantly decreased ($p<0.001$). A significant decrease in serum glucose and insulin was observed in the MKR-treated group than the toxicant control ($p<0.001$). HOMA-IR was significantly decreased ($p<0.001$) and HOMA-IS and FGIR were significantly increased than the toxicant control ($p<0.001$) on treatment with MKR. Similar changes were observed in the metformin-treated group ($p<0.001$ for serum glucose, insulin, HOMA-IR, HOMA-IS, and FGIR). Glucose levels in the metformin-treated group were significantly lower than the MKR-treated group on day 28 ($p<0.01$). Metformin treatment showed significantly higher HOMA-IS as compared to the MKR-treated group ($p<0.01$). The results are depicted in Figure 1.

Serum lipid parameters

Dexamethasone treatment significantly elevated serum cholesterol, triglyceride, LDL, and VLDL levels and significantly reduced serum HDL levels than the normal control ($p<0.001$). A significant decrease in serum cholesterol, triglyceride, LDL, and VLDL levels was observed in the MKR-treated group than the negative control ($p<0.001$), whereas the level of serum HDL was significantly increased ($p<0.001$). Similar changes were observed in the metformin-treated group ($p<0.001$ for serum cholesterol, HDL, LDL, VLDL, and triglycerides). No significant difference was seen between the MKR-treated group and the metformin-treated group in serum cholesterol levels. MKR-treated group significantly reduced the levels of serum triglycerides, LDL, and VLDL as compared to the metformin-treated group ($p<0.001$ for triglyceride and VLDL levels and $p<0.05$ for LDL levels). Also, MKR treatment significantly increased the levels of HDL as compared to the metformin-treated group ($p<0.01$). The results are depicted in Figure 2.

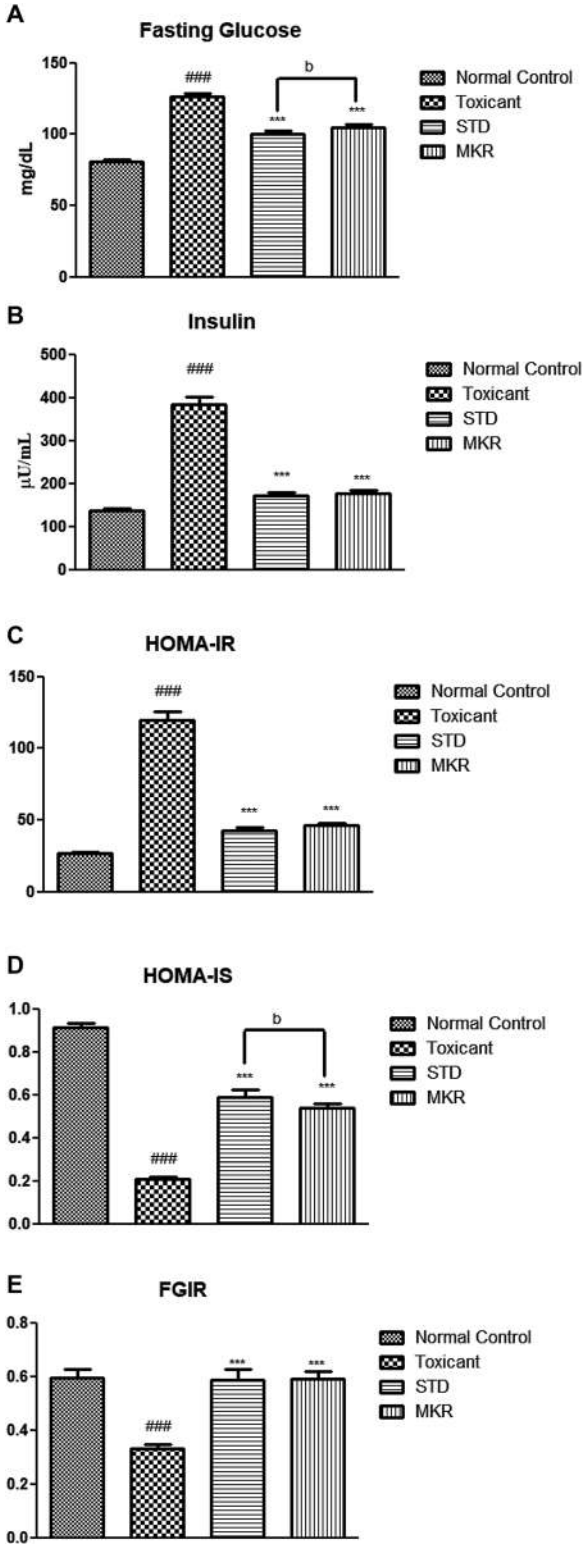

Figure 1: Effect of MKR on (A) fasting serum glucose and (B) insulin levels (C) HOMA-IR (D) HOMA-IS (E) FGIR. Values are expressed as Mean ± SD (n=6). Statistical analysis was carried out by one-way ANOVA followed by Bonferroni post hoc multiple comparison test. ###p<0.001 v/s normal control, *p<0.05 v/s toxicant control and ***p<0.001 v/s toxicant control; bp<0.01 v/s MKR group.

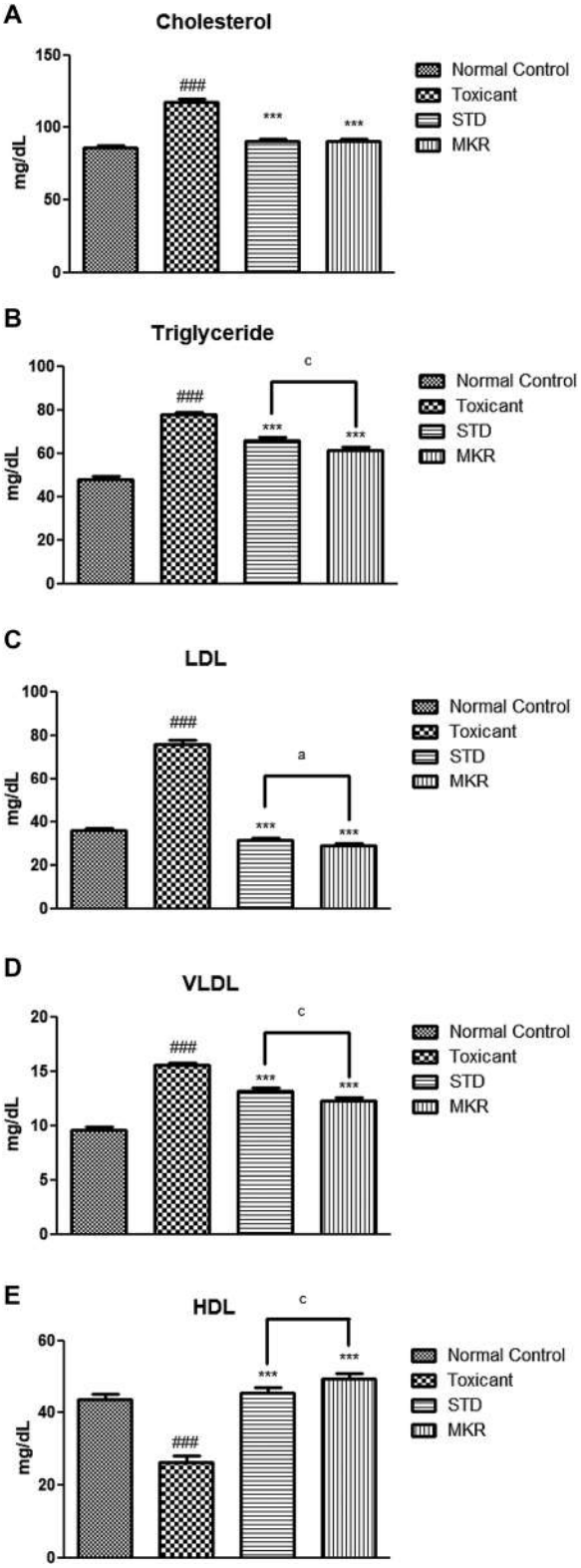

Figure 2: Effect of MKR on (A) serum cholesterol (B) triglycerides (C) LDL (D) VLDL (E) HDL (mg/dL). Values are expressed as Mean ± SD (n=6). Statistical analysis was carried out by one-way ANOVA followed by Bonferroni post hoc multiple comparison test. ###p<0.001 v/s normal control, ***p<0.001 v/s toxicant control, ap<0.05, cp<0.001 v/s standard control.

OGTT

Dexamethasone treatment significantly elevated the value of OGTT than the normal control ($p<0.001$). Treatment with MKR significantly decreased OGTT values as compared to the toxicant control ($p<0.01$). Similar changes were observed in the metformin treated group ($p<0.01$). No significant difference was observed between MKR- and metformin-treated groups. The results are depicted in Figure 3.

MDA and GSH levels

Dexamethasone treatment significantly elevated the levels of MDA and significantly decreased the levels of GSH than the normal control ($p<0.001$). Treatment with MKR significantly decreased MDA levels and significantly revitalized GSH levels than the toxicant control ($p<0.001$). Similar changes were observed in the metformin-treated group ($p<0.05$ for GSH and $p<0.001$ for MDA). The levels of MDA were found to be significantly lower in the MKR-treated group as compared to the metformin-treated group ($p<0.001$). MKR treatment revitalized the GSH levels that were found to be significantly higher than the metformin-treated group ($p<0.001$). The results are depicted in Figure 4.

Histopathological observations

Figures 5–7 depict cytoprotective role of MKR.

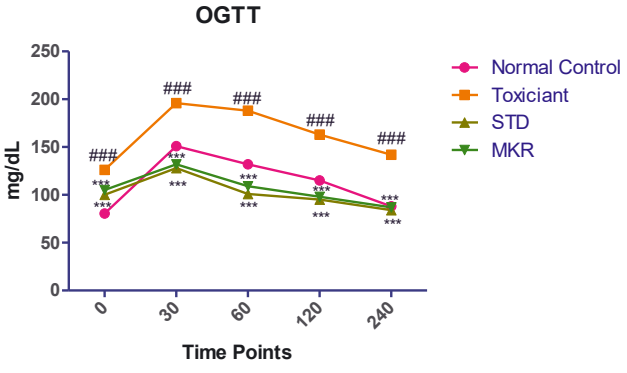

Figure 3: Effect of MKR in OGTT (mg/dL). Values are expressed as Mean ± SD (n=6). Statistical analysis was carried out by two-way ANOVA followed by Bonferroni post hoc multiple comparison test. ###p<0.001 v/s normal control and ***p<0.001 v/s toxicant control.

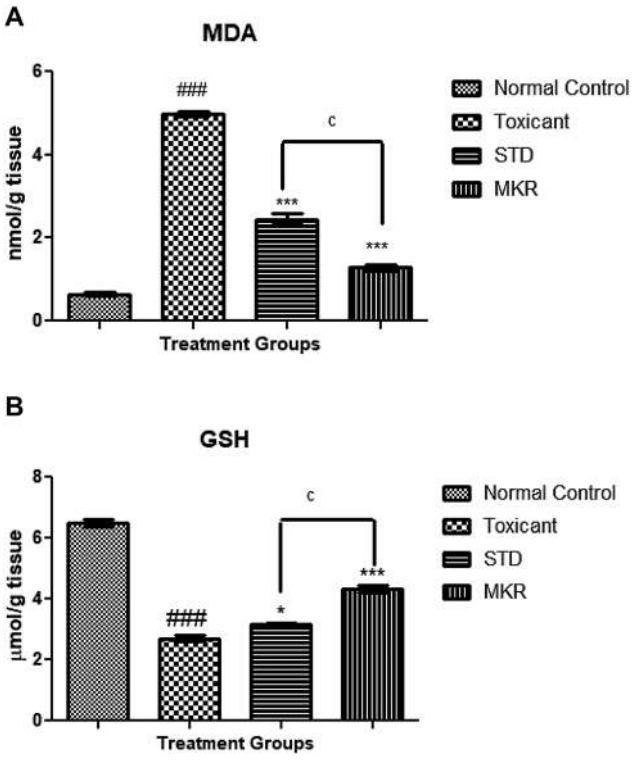

Figure 4: Effect of MKR on (A) MDA (nmol/g tissue) and (B) GSH (µmol/g tissue) levels. Values are expressed as Mean ± SD (n=6). Statistical analysis was carried out by one-way ANOVA followed by Bonferroni post hoc multiple comparison test. ###p<0.001 v/s normal control and *p<0.05, ***p<0.001 v/s toxicant control, and cp<0.001 v/s standard control.

Discussion

T2DM is characterized by hyperglycemia and development of IR, which is strongly correlated with metabolic disorders. IR causes dyslipidemia leading to an imbalance in cholesterol and triglyceride levels [14] and increases the risk of developing obesity [15]. Insulin is responsible for the inhibition of hormone sensitive lipase (HSL), which keeps the free fatty acid (FFA) levels in blood under control. Impaired HSL inhibition increases plasma FFA leading to their accumulation in peripheral tissues causing obesity. This accumulation of FFA also inhibits effective glucose uptake by cells inhibiting insulin pathway and further exacerbating IR [16]. Furthermore, it is studied that free fatty acids induce IR by reducing glycogenesis and glucose oxidation in muscles by 50% [17].

Metformin is the drug of choice for the management of IR-associated T2DM as well as other conditions like obesity and hyperlipidemia. Metformin acts via various modes of

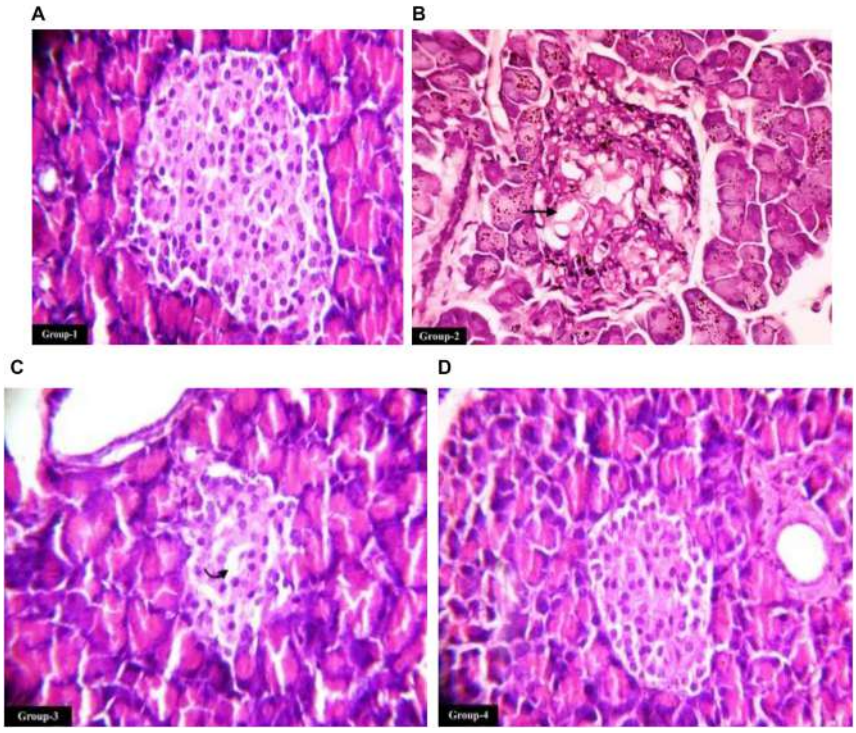

Figure 5: Histology of the pancreas on treatment with dexamethasone, metformin, and MKR. A represents normal control; B represents toxicant control; C represents standard control; and D represents the MKR-treated group. Black arrow indicates vacuolation.

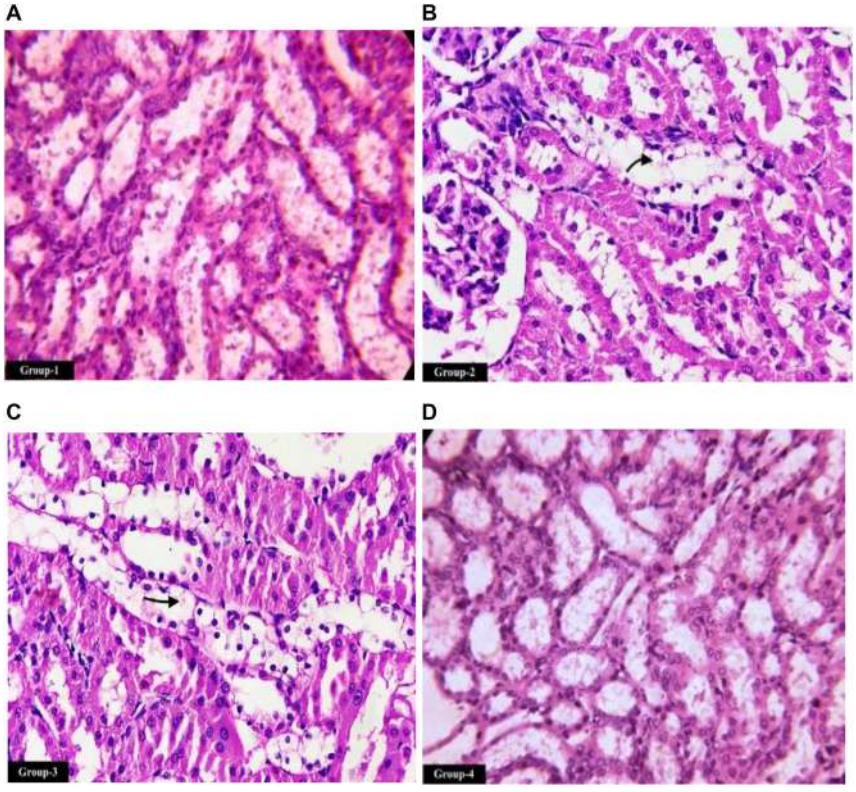

Figure 6: Histology of the kidney on treatment with dexamethasone, metformin, and MKR. A represents normal control; B represents toxicant control; C represents standard control; and D represents the MKR-treated group. Black arrow indicates vacuolation.

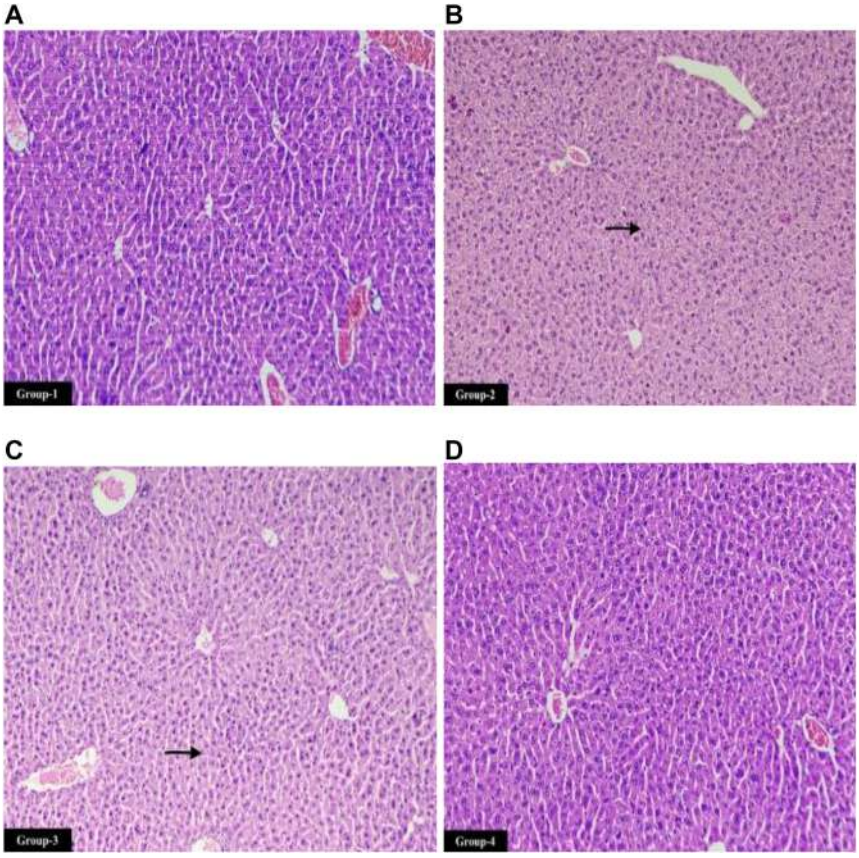

Figure 7: Histology of the liver on treatment with dexamethasone, metformin, and MKR. A represents normal control; B represents toxicant control; C represents standard control; and D represents the MKR-treated group. Black arrow indicates degeneration.

action such as suppressing gluconeogenesis, inducing GLUT-4-mediated glucose uptake, decreasing glucose absorption from the gastrointestinal tract, and increasing fatty acid oxidation [18]. It also increases insulin sensitivity and decreases hyperglycemia. Clinically, metformin has been reported to control plasma glucose and lipid profiles [19] and decrease HOMA-IR and improve Homeostasis Model Assessment for β-cell function without causing hyperinsulinemia [20,21]. Thus, due to its proven clinical efficacy in the management of IR, metformin was used as the standard in the present study.

IR associated with T2DM is defined as impaired ability of insulin to control nutrient partitioning in target organs. In adipose tissue due to IR, insulin fails to restrain lipolysis and decrease glucose uptake. In liver, IR promotes hepatic gluconeogenesis and glycogenolysis. In this study, on administration of dexamethasone, similar effects were observed in animals representing induction of IR.

Chronic administration of dexamethasone impairs insulin-mediated glucose metabolism, alters insulin stimulated glucose transporters like GLUT-4, and impairs insulin-induced increase in muscle blood flow leading to development of IR, which is similar to natural IR [22]. Here, dexamethasone (1.5 mg/kg/day *i.p.* for 28 days) was used to induce IR [8]. In this study, dexamethasone-induced IR was evidenced by significant increase in fasting serum glucose and insulin levels as compared to the normal control. In OGTT, the left side shift in graph of serum glucose (mg/dl) vs series of time points is a clear indication of development of IR. Dexamethasone-induced hyperglycemia was found to be prevented on MKR treatment. Although, fasting serum glucose levels in the MKR-treated groups were significantly lower than toxicant control, they were found to be slightly higher than that of metformin-treated group. However, MKR treatment showed a faster onset of action as compared to metformin. Importantly, no significant difference was observed in the OGTT value between MKR- and metformin-treated groups showing that both MKR and metformin were equally effective in clearing the excess glucose load in this model. The probable mechanism of action could be attributed to the individual ingredients of MKR and their synergistic effects. *Tinospora cordifolia* is known for its anti-diabetic potential. It not only maintains glycemic control but also possesses multiple target actions obliterating the

complex diabetic pathology [23]. Vasant Kusumakar Rasa is a generic *Ayurved* medication that is widely used by *Ayurved* practitioners. It has an anti-hyperglycemic action and is documented to be useful for the management of T2DM and its complications like diabetic retinopathy and neuropathy [24, 25]. *Pterocarpus marsupium* is reported to stimulate insulin secretion and glucose uptake in muscles and pancreatic tissues. *Enicostemma littorale* is known to have a hypoglycemic effect [26]. The aqueous extract of *Aegle marmelos* is also reported for its hypoglycemic effect [27]. Processed Zinc in MKR is also documented for its anti-diabetic activity [28, 29].

Insulin is an important hormone not only for maintaining glucose levels in blood but also maintaining the lipid profile. Treatment with MKR significantly reduced the levels of serum insulin as compared to the dexamethasone-treated group, thus controlling hyperinsulinemia. Hyperinsulinemia is strongly correlated to T2DM and its associated conditions as the insulin receptors are desensitized in IR. In present study, to evaluate extent of insulin sensitization on treatment with MKR various insulin indices like HOMA-IR, HOMA-IS, and FGIR were determined. These indices are important to assess the functioning of beta cells of pancreas and hepatic glucose output that is mediated by insulin. MKR treatment improved these indices i.e. decrease in HOMA IR indicated decreased IR and increase in HOMA-IS and FGIR indicated improved whole-body insulin sensitivity. Thus, MKR treatment showed overall attenuation of IR. MKR contains *Curcuma longa* with anti-hypertensive effect and is also effective in ameliorating hepatic steatosis and IR in experimental models [30, 31]. Ellagic acid in *Emblica officinalis* present in MKR has been reported to stimulate the beta cells of pancreas to secrete insulin and decrease systemic glucose levels [32]. It is also documented to prevent IR by checking the lipid levels [33].

To address IR-associated disorders like hyperlipidemia, atherosclerosis, PCOS, etc. lipid parameters like serum cholesterol, LDL, VLDL, HDL, and triglycerides were evaluated. Development of IR leads to increased hormone-sensitive lipase activity of adipose tissue and decrease in lipoprotein lipase activity, resulting in enhanced mobilization of fatty acids from adipocytes and increased hepatic synthesis of triglycerides, which are then released into the bloodstream as VLDL cholesterol [34]. Also, in condition like hypertriglyceridemia, enzyme Cholesterol Ester Transfer Protein transfers triglycerides from VLDL cholesterol to HDL which are hydrolyzed rapidly thus decreasing HDL blood levels [35]. In this study, dexamethasone-treated group exhibited significant decrease in HDL and increase in VLDL levels. Significant hypercholesterolemia observed in dexamethasone-treated group might be the result of hypertriglyceridemia in liver causing increased expression of sterol regulatory element-binding protein (SREBP) which regulates the trapping and synthesis of cholesterol [34].

Dyslipidemia is a major factor responsible for the development of IR in T2DM. Excessive production of VLDL in the liver and increased adipocyte free fatty acid flux from lipolysis under IR also develops chances of tissue and circulating lipid abnormalities. As insulin plays critical role in the inhibition of hepatic VLDL formation in the liver and lipolysis in adipose tissues, recovery of insulin action in these tissues is one of the key strategies to deal with abnormal lipid profile in T2DM condition [36].

In this study, MKR treatment significantly reduced triglycerides, cholesterol, LDL and increased HDL levels. These results could thus reflect the ability of MKR to improve the insulin sensitivity of tissue thereby reducing hormone sensitive lipase activity and enhancing lipoprotein lipase activity, resulting in reduced lipolysis. MKR thus possesses a significant anti-hyperlipidemic effect. Importantly, improvement in lipid profile was more significant with MKR in contrast to the metformin-treated group. Lowering of triglyceride, LDL, VLDL and increase in the levels of serum HDL was significantly greater in MKR-treated group than the metformin-treated group. This is of peculiar importance because metformin is conventional drug of choice for the management of IR-associated conditions like T2DM and obesity whereas this study showed that MKR exhibits better lipid profile stabilizing effect than metformin. *C. longa* is reported to improve lipid profile and decrease the body mass index in T2DM-dyslipidemic patients [37, 38]. *Pterocarpus marsupium* is reported to significantly reduce the body weight, abdominal circumference, blood sugar, and serum triglyceride levels in an experimental model of metabolic syndrome [39]. Moreover, *Enicostemma littorale*, has an anti-hyperlipidemic activity as well as an anti-hyperinsulinemic activity [26]. The aqueous extract of *Enicostemma littorale* is reported to increase insulin sensitivity, normalize dyslipidemia, and provide nephroprotection in diabetic rats [40]. *E. officinalis* is known to decrease LDL, cholesterol, increase HDL and is also documented to decrease triglycerides thereby preventing dyslipidemia in ovaricetomized female albino rats [33]. Thus, observed anti-hyperlipidemic effect of MKR is attributed to the synergistic activity of these constituents.

Here, dexamethasone administration was also found to induce oxidative stress as evidenced by increased MDA and decreased GSH levels in pancreatic tissue. Histopathological degeneration of liver, kidneys, and pancreatic tissues observed on dexamethasone administration is

indicative of its detrimental effects. Hyperglycemia induces oxidative stress, by impairing level of antioxidant enzymes [41]. Increased oxidative stress induces and aggravates IR [42]. Thus, to probe effect of MKR treatment on oxidative stress markers, GSH and MDA, were estimated in the pancreatic tissue. In MKR-treated group, significant restoration of GSH levels and reduction in MDA levels than the dexamethasone-treated group indicate potent antioxidant potential. Interestingly, antioxidant effect exhibited by MKR is significantly greater than metformin. This significant antioxidant effect of MKR can thus be attributed to its components like *Enicostemma littorale* documented for anti-inflammatory and antioxidant activity [26]. Vasant Kusumakar Rasa also known for its antioxidant effect. The antioxidant and anti-inflammatory effects of *E. officinalis*, *C. longa*, and *Aegle marmelos* are well documented to regulate free radical scavenging activity and also for anti-hyperglycemic effect. Thus, antioxidant effect of MKR offers additional therapeutic potential over conventional drugs to overcome IR in T2DM [43–45].

To study the histopathological changes associated with dexamethasone and MKR treatment, we performed H&E staining. Degeneration was seen in the liver, kidney, and pancreatic tissues on dexamethasone treatment. MKR treatment prevented this degeneration and protected tissues from apoptosis. Interestingly, *Enicostemma littorale* is documented to have hepatoprotective activity [26], which can be contributed to the observed protective effect of MKR on liver [40].

Thus, this study supports the fact that IR results in hyperglycemia and hyperlipidemia thereby increasing risk of T2DM and CVDs. MKR contains active constituents, which impart ability to prevent dyslipidemia and any further complications due to development of IR. Improved lipid profile on treatment with MKR is an indication of therapeutic usefulness of MKR in IR-associated disorders like T2DM, obesity, hyperlipidemia, PCOS, hypertriglyceridemia, atherosclerosis, etc. Thus, this study confirms the effectiveness of MKR as an anti-diabetic formulation not only to curb hyperglycemia associated with T2DM but also to prevent potential risk factors like dyslipidemia associated with IR and T2DM.

Conclusion

Treatment with MKR exhibits a significant role in preventing IR and reducing the risk of T2DM. MKR significantly reduced serum fasting glucose and insulin levels and exhibited significant improvement in insulin indices like HOMA-IR, HOMA-IS and FGIR. MKR also improved lipid parameters and especially promoted increase in HDL levels. OGTT confirmed its blood glucose lowering action which is comparable with standard metformin. Most importantly MKR reduced oxidative stress in pancreatic tissue. It can be concluded that MKR has a good therapeutic potential to prevent IR and improve insulin sensitivity to reduce hyperglycemia. Thus, MKR can be used to treat IR-associated conditions like T2DM. MKR is also found to be significantly better than metformin in improving lipid profile and restoring levels of antioxidant enzyme in pancreas.

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Competing interests: Two authors are from Shree Dhootapapeshwar Limited, which have also funded the study. The other authors declare no conflict of interest.
Ethical statement: The study protocol was approved by the Institutional Animal Ethics Committee (Protocol number-KMKCP/IAEC/181903).
Employment or leadership: None declared.
Honorarium: None declared.

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