Original Article

Assessment of Antidiabetic Activity of Combination of *Murraya koenigii* Leaves Extract and *Vitis vinifera* Seeds Extract in Alloxan-induced Diabetic Rats

Abstract

Background: *Murraya koenigii* is a well-known curry leaf tree. Its leaves are used as a spice in food recipe in India. Its related antidiabetic activity is attributed to alpha glucosidase activity of carbazole alkaloids. The proanthocyanidins present in *Vitis vinifera* contribute to its hyperglycemic activity through antioxidant effect and preservation of β-cell function. **Aims and Objective:** The aim of this study was to assess antidiabetic effect of the combination of *M. koenigii* leaves extract and *V. vinifera* seeds extract. **Materials and Methods:** A total of 36 animals were randomly selected for the study and were divided in six different groups: control group, alloxan (130 mg/kg; intraperitoneal [i.p.]) treated group, alloxan (130 mg/kg) treatment + *M. koenigii* leaves extract (300 mg/kg; per oral [p.o.]) treated group, alloxan (130 mg/kg) i.p. treatment + *V. vinifera* seeds extract (200 mg/kg; p.o.) treated group, alloxan (130 mg/kg) + *M. koenigii* leaves extract (150 mg/kg; p.o.) treatment + *V. vinifera* seeds extract (100 mg/kg; p.o.) treated group, and alloxan (130 mg/kg) treatment + glibenclamide (5 mg/kg; i.p.) treated group. Treatment was given for 21 days after induction of diabetes. **Result:** The combination treated group showed a significant reduction in serum glucose levels when compared to individual test extracts. It also attenuated the elevated activity of alkaline phosphatase enzyme in diabetic rats as compared with the healthy controls. The combination treatment showed reversal of the serum glutamic oxaloacetic transaminase ($P < 0.001$) and serum glutamic pyruvic transaminase ($P < 0.001$) levels. It also significantly decreased cholesterol level to near normalcy ($P < 0.001$). **Conclusion:** The findings of this study suggest additive antidiabetic effect of the combination of *M. koenigii* leaves extract and *V. vinifera* seeds extract.

Keywords: *Alloxan-induced diabetes, antidiabetic, Murraya koenigii, Vitis vinifera*

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Introduction

Diabetes is considered as one of the major causes of morbidity and mortality affecting young and middle-aged population.[1] According to International Diabetes Federation, the total number of people across the world with diabetes is projected to rise from 387 million in 2014 to 592 million by 2035.[2] The prolonged use of present oral hypoglycemic agents and insulin is associated with development of resistance and various side effects. The traditional herbal options may help in fulfilling these unmet needs.[3] There are various herbs having proven antidiabetic effect such as *Memordica charantia*, *Eugenia jambolana*, *Trigonella foenum graecum*, *Embilca officinalis*, *Azadirachta indica*, *Phaseolus vulgaris*, and *Gymnema sylvestere*.[3] *Murraya koenigii* Spreng is from Rutaceae family (Citrus family). It is commonly known as curry leaf tree.[4-6] The *M. koenigii* (curry leaf) plant is widely used as herb, spice, condiments, and is also used to treat various types of diseases in Indian traditional system. Its related antidiabetic activity is attributed to the alpha glucosidase activity of carbazole alkaloids. Alpha glucosidase inhibitory activity prevents digestion of carbohydrates and thereby reduceslucose absorption.[5,6] *Vitis vinifera* (grape) is a plant native to Asia, Mediterranean region, and North Africa. The hyperglycemic activity of grape seeds could be attributed to presence of proanthocyanidines. Proanthocyanidines, also called potent antioxidant, exert antidiabetic effects by preserving β-cell function.[7]

M. koenigii leaves[4-6]and *V. vinifera* seed[7-9] have shown antidiabetic activity individually in separate studies. *M. koenigii* (leaves) contains multiple bioactive agents (e.g., carbazole alkaloids, flavonoids, and tannins),

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which are known for their potent antihyperglycemic action by regeneration of beta cells in pancreas and reducing blood sugar levels.[4,5] Active chemical constituent like mahanine present in *M. koenigii* also showed alpha-glucosidase inhibitory activity, which prevents digestion of carbohydrates,[10] whereas *V. vinifera* (seeds) possesses antioxidant activity and shows protective effect on pancreatic islets against oxidative stress in diabetes.[7-9] As *M. koenigii* leaves and *V. vinifera* seeds have different mechanisms and site of actions, the combination of these may produce synergy in antidiabetic action. Therefore, the aim of this study was to assess the antidiabetic effect of their combination in alloxan-induced diabetic rats.

Materials and Methods

Experimental animals

Healthy male Wistar rats (body weight range 200–220 g) were considered. Animal were housed in cages lined with husk. The maintained environmental conditions include temperature 25 ± 2°C and 12:12 h light:dark cycle. The rats had free access to standard pellet diet *ad libitum* and water. The experimental protocols were approved by the Institutional Animal Ethics Committee (Approval number CPCSEA/IAEC/BNCP/P-27/2014).

Chemicals

Alloxan was purchased from Spectrochem, Mumbai, India, and ethanol was purchased from S D Fine Chem, Mumbai, India. Erba diagnostic kits for glucose, cholesterol, urea, creatinine, bilirubin, alkaline phosphatase (ALP), serum glutamic oxaloacetic transaminase (SGOT), and serum glutamic pyruvic transaminase (SGPT) were purchased from Nobel Diagnostics, Mumbai, India.

Plant materials and authentication

The curry leaves (*M. koenigii* leaves) were procured from local market, Mumbai. Leaves were authenticated by Department of Botany, Mithibai College, Vile Parle (West), Mumbai (Letter no. MIT 0063). The *V. vinifera* seed extract (grape seed extract) was procured from Navchetna Kendra, Delhi, India. It was provided with certificate of analysis from Navchetna Kendra, Delhi, India.

Preparation of extract (Murraya koenigii leaves)

The *M. koenigii* leaves were dried in air at room temperature. The dried leaves were grounded into fine powder in electrical grinder. The powdered material was used for the preparation of extract. The extraction was carried out by soxhlet extraction method using ethanol as a solvent. For extraction, 20 g of powdered leaves was packed in a thimble containing filter paper and extracted with ethanol 90% (60°C–70°C) in soxhlet apparatus for period till all the crude substances were extracted. The extract thus obtained was concentrated with the help of rotary vacuum evaporator. The extract was then dried at 40°C.[11]

Preliminary phytochemical evaluation

Curry leaf (*M. koenigii* leaves) extract was subjected to qualitative phytochemical evaluation for identification major classes of active constituents.[12] Chemical tests helped in qualitative estimation of alkaloids (Dragendorff's test), amino acids (ninhydrin test), carbohydrates (Molisch test), flavonoids (Shinoda test, alkaline reagent test, and zinc hydrochloride test), glycosides, phenolic compound, steroids, and so on.[12]

Test samples preparations

M. koenigii leaves extract, *V. vinifera* seeds extract, and glibenclamide drug were suspended in 0.5% sodium carboxymethyl cellulose separately. Saline (0.9% wt/vol NaCl) was used to prepare 0.5% sodium carboxymethyl cellulose.

Experimental design and treatment

Animals were randomly divided in six different groups ($n = 6$ in each group): Group A: vehicle-treated group intraperitoneal (i.p.) treatment (control group), Group B: alloxan (130 mg/kg; i.p.) (diabetes control)[13,14] treated group, Group C: alloxan (130 mg/kg; i.p.) treatment + *M. koenigii* leaves extract (300 mg/kg; per oral [p.o.])[5,15,16] treated group, Group D: alloxan (130 mg/kg; i.p.) + *V. vinifera* seeds extract (200 mg/kg; p.o.)[7,8,17-19] treated group, Group E: alloxan (130 mg/kg; i.p.) + *M. Koenigii* leaves extract (150 mg/kg; p.o.) treatment + *V. vinifera* seeds extract (100 mg/kg; p.o.) treatment (combination treated), and Group F: alloxan (130 mg/kg; i.p.) treatment + glibenclamide (5 mg/kg; i.p.)[20] treated group (standard treatment).

Induction of diabetes

The control group (Group A) was treated with normal saline. Diabetes was induced in 4 h fasted animals using a single-dose injection of alloxan (130 mg/kg; i.p.). The same volume of normal saline was administered to diabetic control group. Animals with more than 250 mg/dL of serum glucose levels on third day of alloxan treatment were considered for further study (Groups B, C, D, E, and F). On the third day of successful diabetes induction, treatments with test substances were started and serum glucose levels analyzed.[13] Therefore, the third day from alloxan administration was Day 1 of treatment schedule.

Acute effect

Acute hypoglycemic studies were performed in overnight fasted diabetic rats on Day 1 of treatment schedule. After receiving treatment described under "Experimental design and treatment" section, blood was withdrawn from retro-orbital plexus at the intervals of 0, 1, 2, 4, and 6 h and serum glucose levels were determined on Day 1 of 21-day treatment schedule. Blood samples were collected from retro-orbital plexus of animals.

Animals of each group were fasted for 12 h and then slightly anesthetized by diethyl ether inhalation while collecting

blood from retro-orbital plexus. Serum was separated by centrifugation at 3000 rpm for 10 min. The separated serum was used for the estimation of glucose and other biochemical parameters.

Subacute effect

Animals received treatment daily for 21 days.[21,22] Serum glucose level was determined at the intervals of Days 1, 7, 14, and 21 of treatment schedule. Other biochemical parameters were evaluated on Day 21 of treatment schedule.

Estimation of biochemical parameters

Serum glucose levels and levels of other biochemical parameters (cholesterol, urea, creatinine, bilirubin, ALP, SGOT, and SGPT) were measured by commercially supplied Erba diagnostic kits (Erba Mannhein, Germany) using Erba chem 7 clinical chemistry analyzer (Erba Mannhein).

Oral glucose tolerance test

Oral glucose tolerance test was performed on Day 21 of treatment. Animals received glucose (2 g/kg body weight; p.o.) 20 min after the administration of respective extract/drug treatment. Blood samples were collected before glucose administration and at 30, 60, and 120 min after glucose loading for immediate measurement of blood glucose levels.

Statistical analysis

All values are expressed as mean ± standard error mean (SEM). The effects of test extracts on boy weight, feed intake, liquid intake, blood glucose levels, and biochemical parameters were determined using one-way analysis of variance test followed by Tukey's honest test. A value of $P < 0.05$ was considered to statistically significant. Data were analyzed using GraphPad Instat 3 software (GraphPad Software, Inc. 2365 Northside Dr. Suite 560 San Diego, CA 92108, USA).

Results

Preliminary phytochemical evaluation

The yield of alcoholic leaf extract of *M. koenigii* was 10.2%. The phytochemical analysis revealed the presence of alkaloids, carbohydrates, flavonoids, proteins, and glycosides in curry leaf extract [Table 1]. The certificate of analysis (provided by supplier) of *V. vinifera* seed extract (grape seed extract) indicated the presence of oligomeric proanthocyanidines.

Table 1: Chemical tests of alcoholic leaf extract of *Murraya koenigii*

Chemical tests	Inference
Alkaloids	+
Amino acids	–
Protein	+
Carbohydrate	+
Flavonoids	+
Glycosides	+
Phenolic compounds	+
Steroids	+
Gums/mucilage	–

Acute effect

Group B showed a significant increase in serum glucose levels ($P < 0.001$) at all the time intervals as compared with Group A [Table 2]. Group C showed a significant decrease in serum glucose levels at 4 h ($P < 0.05$) and 6 ($P < 0.01$) h time intervals as compared with Group B. Group D showed a significant decrease in serum glucose levels at 2 h ($P < 0.05$), 4 h ($P < 0.001$), and 6 h ($P < 0.001$) time intervals as compared with Group B. With the exception at 0-h time interval, Groups E and F showed a significant decrease in serum glucose levels at all the time intervals as compared with Group B. Group E showed a significant decrease in serum glucose levels ($P < 0.05$) only at 6-h time interval as compared with Group C [Table 2].

Subacute effect

Group B (alloxan treated) showed a significant increase ($P < 0.001$) in fasting serum glucose levels as compared with Group A [Table 3]. Group B showed a significant decrease in fasting serum glucose levels as compared with Groups C, D, and E at 14th and 21st days of treatment interval [Table 3]. Group E also showed similar benefits at 7th day of treatment interval. Groups C ($P < 0.01$) and D ($P < 0.05$) showed a significant reduction in serum glucose levels as compared with Group E on 21st day of treatment (combination treated) [Table 3]. Group F (glibenclamide) showed a significant decrease in serum glucose levels as compared with Group B at 7th, 14th and 21st days of treatment interval [Table 3]. However, a similar reduction of serum glucose levels observed with Group F (glibenclamide) was not found statistically significant as compared with Group E (combination treated) [Table 3].

Oral glucose tolerance test

Group B showed a significant increase ($P < 0.001$) in serum glucose levels at all the time intervals as compared with Group A [Table 4]. Groups C, D, E, and F showed a significant decrease ($P < 0.001$) in serum glucose levels at all time intervals than Group B. Group E showed a significant ($P < 0.001$) decrease in serum glucose levels at all time intervals as compared with Groups C and D separately. Group F showed a significant ($P < 0.001$) decrease in serum glucose levels as compared with Group E [Table 4].

Biochemical parameters

Group B showed a significant increased ($P < 0.001$) levels of cholesterol, urea, and creatinine in serum as compared with Group A [Table 5]. Groups C, D, E, and F showed a significant decrease in serum cholesterol, urea, and creatinine levels as compared with Group B. Group E showed a significant decrease in serum cholesterol and urea levels as compared with Groups D ($P < 0.01$) and C ($P < 0.001$), respectively. Group F showed a significant decrease in serum cholesterol

Table 2: Acute effect of treatment on fasting serum glucose levels (mg/dL)

Group	Serum glucose levels (mg/dL)				
	0 h	1 h	2 h	4 h	6 h
Group A (vehicle control)	113.02 ± 5.632	115.18 ± 4.931	114.1 ± 4.789	113.92 ± 4.312	113.38 ± 4.644
Group B (alloxan-induced diabetes)	410.95 ± 5.699$^{\$\$\$}$	414.78 ± 5.315$^{\$\$\$}$	414.97 ± 4.868$^{\$\$\$}$	416.05 ± 4.565$^{\$\$\$}$	417.68 ± 3.872$^{\$\$\$}$
Group C (alloxan + *Murraya koenigii*)	398.5 ± 7.62	396.1 ± 7.003	393.1 ± 6.85	387.8 ± 6.5298*	382.47 ± 5.392**
Group D (alloxan + *Vitis vinifera*)	392.2 ± 9.343	389.7 ± 9.153	385.6 ± 9.492*	375.1 ± 8.997***	372.17 ± 8.972***
Group E (alloxan + combination treated)	389.38 ± 7.472	378.37 ± 7.739**	376.82 ± 5.319**	367.1 ± 5.299***	353.38 ± 5.79***@
Group F (alloxan + glibenclamide)	384.98 ± 6.749	376.57 ± 6.341**	369.97 ± 6.37***	360.9 ± 6.146***	326.7 ± 9.315***

The values are expressed as mean ± SEM and $n = 6$. One-way analysis of variance followed by Tukey's honest test. Significance is denoted by $^{\$\$\$}P < 0.001$ as compared with Group A
*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ as compared with Group B
@$P < 0.01$ as compared with Group C

Table 3: Subacute effect of combination on fasting serum glucose levels in alloxan-induced diabetic rats

Group	Serum glucose levels (mg/dL)			
	Day 1	Day 7	Day 14	Day 21
Group A (vehicle control)	113.02 ± 5.63	112.17 ± 4.39	106.43 ± 4.39	107.48 ± 3.82
Group B (alloxan-induced diabetes)	410.95 ± 5.7$^{\$\$\$}$	416.55 ± 7.59$^{\$\$\$}$	425.68 ± 7.6$^{\$\$\$}$	434.23 ± 6.76$^{\$\$\$}$
Group C (alloxan + *Murraya koenigii*)	398.5 ± 7.62	377.33 ± 5.92	332.88 ± 5.42*	251.85 ± 8.05**
Group D (alloxan + *Vitis vinifera*)	392.2 ± 9.34	364.3 ± 9.26	302.92 ± 9.13*	216.57 ± 6.38**
Group E (alloxan + combination treated)	389.38 ± 7.42	346.1 ± 6.01*	274.68 ± 5.66**	157.15 ± 5.23***@@#
Group F (alloxan + glibenclamide)	384.98 ± 6.75	314.22 ± 7.63**	215.95 ± 8.87***	123.77 ± 4.41***

The values are expressed as mean ± SEM and $n = 6$. One-way analysis of variance followed by Tukey's honest test. Significance is denoted by $^{\$\$\$}P < 0.001$ as compared with Group A
*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ as compared with Group B
$^{@@}P < 0.01$ as compared with Group C
$^{\#}P < 0.05$ as compared with Group D

Table 4: Effect of combination in oral glucose tolerance test in diabetic rats

Group	Serum glucose levels (mg/dL)			
	0 min	30 min	60 min	120 min
Group A (vehicle control)	109.48 ± 3.82	135.33 ± 3.94	127.97 ± 3.57	114.2 ± 3.03
Group B (alloxan-induced diabetes)	430.63 ± 6.76$^{\$\$\$}$	450.18 ± 5.19$^{\$\$\$}$	454.28 ± 5.27$^{\$\$\$}$	459.35 ± 4.37$^{\$\$\$}$
Group C (alloxan + *Murraya koenigii*)	238.4 ± 6.05***	265.7 ± 5.36***	258.88 ± 5.75***	252.23 ± 5.25***
Group D (alloxan + *Vitis vinifera*)	214.75 ± 6.38***	244.35 ± 3.46***	239.34 ± 2.89***	228.3 ± 3.67***
Group E (alloxan + Combination treated)	153.67 ± 5.23***@@@###	166.37 ± 3.93***@@@###	161.03 ± 4.36***@@@###	159.32 ± 4.47***@@@###
Group F (alloxan + glibenclamide)	119.98 ± 4.41***!!	134.93 ± 4.21***!!!	128.13 ± 3.96***!!!	122.45 ± 4.01***!!!

The values are expressed as mean ± SEM at $n = 6$. One-way analysis of variance followed by Tukey's honest test. Significance is denoted by $^{\$\$\$}P < 0.001$ as compared with Group A
*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ as compared with Group B
$^{@@}P < 0.01$ as compared with Group C
$^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ as compared with Group D
$^{!}P < 0.05$, $^{!!}P < 0.01$, and $^{!!!}P < 0.001$ as compared with Group E

($P < 0.001$) and creatinine ($P < 0.01$) levels as compared with Group E [Table 5].

Group B showed a significant ($P < 0.001$) increase in SGOT, SGPT, bilirubin, and ALP serum levels as compared with Group A [Table 5]. Group C showed a significant decrease in SGOT ($P < 0.01$), bilirubin ($P < 0.05$), and ALP ($P < 0.05$) levels as compared with Group B. Groups D, E, and F showed a significant decrease in SGOT, SGPT, bilirubin, and ALP levels as compared with Group B. Group E showed a significant decrease in SGOT ($P < 0.05$) and SGPT ($P < 0.001$) serum levels as compared with Group C. Group F showed a significant decrease in serum bilirubin levels as compared with Group E [Table 5].

Discussion

Alloxan causes partial destruction of pancreatic β cells, which leads to reduced levels of insulin and consequently resulting

Table 5: Effect on serum biochemical parameters in alloxan-induced diabetic rats

Group	Serum biochemical parameters (mg/dL)						
	Cholesterol	Urea	Creatinine	SGOT	SGPT	Bilirubin	ALP
Group A (vehicle control)	58.75 ± 3.54	35.52 ± 1.93	0.86 ± 0.05	47.62 ± 2.64	47.79 ± 3.21	0.84 ± 0.02	47.3 ± 1.86
Group B (alloxan-induced diabetes)	135.57 ± 4.29$^{\$\$\$}$	154.89 ± 3.53$^{\$\$\$}$	1.51 ± 0.04$^{\$\$\$}$	132.09 ± 4.22$^{\$\$\$}$	135.48 ± 4.13$^{\$\$\$}$	1.78 ± 0.06$^{\$\$\$}$	76.92 ± 2.42$^{\$\$\$}$
Group C (alloxan + *Murraya koenigii*)	98.33 ± 2.33***	85.7 ± 4.53***	1.28 ± 0.03*	110.53 ± 3.08**	123.05 ± 3.95	1.57 ± 0.04*	67.14 ± 1.37*
Group D (alloxan + *Vitis vinifera*)	106.3 ± 4.21***	65.5 ± 4.67***	1.25 ± 0.08**	101.38 ± 4.21***	109.59 ± 3.92**	1.5 ± 0.05**	66.46 ± 1.82**
Group E (alloxan + combination treated)	84.4 ± 3.27***$^{\#\#}$	54.77 ± 3.82***$^{@@@}$	1.18 ± 0.02***	92.93 ± 3.27***$^{@}$	96.39 ± 4.87***$^{@@@}$	1.48 ± 0.04***	64.45 ± 1.6***
Group F (alloxan + glibenclamide)	117.37 ± 4.5*$^{!!!}$	55.83 ± 4.01***$^{@}$	0.89 ± 0.05***$^{!!}$	99.72 ± 2.44***	111.88 ± 3.78**	1.08 ± 0.05***$^{!!!}$	58.68 ± 2.05***

The values are expressed as mean ± SEM and $n = 6$. One-way analysis of variance followed by Tukey's honest test. Significance is denoted by $^{\$\$\$}P < 0.001$ as compared with Group A

$^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ as compared with Group B

$^{@@}P < 0.01$ as compared with Group C

$^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ as compared again Group D

$^{!}P < 0.05$, $^{!!}P < 0.01$, and $^{!!!}P < 0.001$ as compared again Group E

into hyperglycemia.[23,24] The hypoglycemic activity of curry leaves could be due to the presence of carbazole alkaloids, which possess alpha-glucosidase inhibitory property. This intestinal enzyme breaks down complex sugars into glucose and thereby decreased serum glucose levels were observed in the curry-leaf-treated group. Other possible mechanism of action of curry-leaf-treated group could be potentiating insulin secretion from β cells of islets, which leads to reduced blood glucose levels.[21] The hyperglycemic activity of grape seeds could be attributed to presence of proanthocyanidines. Proanthocyanidines have been reported to possess the antioxidant activity. Proanthocyanidines, also called potent antioxidant, can exert antidiabetic effects by preserving β-cell function.[25] The combination of curry leaf extract and grape seed extract showed a significant reduction in serum glucose levels as compared with individual test extracts.

The abnormal high concentration of cholesterol in the blood of diabetes is mainly due to the increase in the mobilization of free fatty acids from the peripheral depots, as insulin inhibits the hormone lipase.[26] Administration of combination of extracts significantly decreased cholesterol level to near normalcy and therefore may reduce the risk of diabetes-associated cardiovascular diseases.

ALP is a liver marker enzyme often used to assess the integrity of plasma membrane and endoplasmic reticulum. Damage to structural integrity of the liver is reflected by an increase in the activity of this enzyme enzymes in the serum, probably as a result of leakage from altered cell membrane structure.[27,28] Therefore, the increase in serum ALP activity in the untreated diabetic rats confirms damage to the plasma membrane, leading to a compromise of membrane integrity. The combination treatment attenuated the elevated activity of ALP enzyme in diabetic rats as compared with the healthy controls.

The transaminases SGOT and SGPT are well-known enzymes used as biomarkers to predict possible toxicity to the liver. Elevation in serum activities of both transaminases as observed in diabetic rats suggested damage to the liver cells as well.[29] The combination treatment also showed benefits in terms of reduction in the levels of these enzymes. Bilirubin is produced from the breakdown and destruction of aged red blood corpuscles and has a diagnostic value.[30,31] Liver health is directly correlated to the levels of bilirubin.[31] In this study, elevated serum levels of bilirubin in diabetic rats may be a result of reduced uptake arising from liver disease. The combination treatment showed reversal of this condition.

The increase in serum levels of urea, creatinine in the alloxan-treated diabetic rats was also observed in this study. Deficiency of insulin and consequent inability of glucose to reach the extrahepatic tissues stimulate gluconeogenesis as an alternative route of glucose supply. This route is sustained by increased proteolysis, which releases free glucogenic amino acids into the plasma that are deaminated in the liver with the consequence of increased urea in the blood. Creatinine is a metabolite of muscle creatine, and its concentration in serum is proportional to the body muscle mass. The amount of creatinine is usually constant; hence, elevated levels indicate diminished renal function only, as it is easily excreted by the kidneys.[29] The combination treatment showed a reduction in the levels of these two metabolites, thereby conferring protection against impairment because of diabetes.

Alteration in the carbohydrate metabolism during post glucose administration can be identified by oral glucose tolerance test.[32] The lower blood glucose levels observed in combination treated group suggest better glucose utilization capacity, thereby indicating increased glucose tolerance. It may be the

result of insulin emission from β cells and improved glucose transport and consumption.

Conclusion

The findings of this study suggest additive antidiabetic effect of the combination of *M. koenigii* leaves extract and *V. vinifera* seeds extract. The combination treatment showed slightly better activity than respective monotherapies. The additive effect of combination treatment may be attributed to the different site and mechanism of actions of the test articles. However, there is a need to further explore the antidiabetic effect of the present combination treatment in different preclinical and clinical settings.

Ethical policy and institutional review board statement

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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Conflicts of interest

There are no conflicts of interest.

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