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ANTIDIABETIC EFFECT OF KERNEL SEEDS EXTRACT OF *MANGIFERA INDICA* (ANACARDIACEAE)

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Background information

Diabetes mellitus is most common disease; the oral antidiabetic is used to treat the diabetes mellitus. The oral antidiabetic agents are causing mild to serious adverse effects during course of the treatment. So in emerging situation needs some alternative system of medicines to treat diabetes mellitus with less adverse effects. Lots of medical plants are distributed throughout India, and those medicinal plants are widely used for treatment of wide variety of diseases. The present study was planned to investigate the possible antidiabetic activity of leaves and kernel seeds extract of *Mangifera indica*.

ABSTRACT

Objective: To study the antidiabetic activity of leaves and kernel seeds extract of *Mangifera indica*.

Material and method: *Mangifera indica* leaves and kernel seeds were extracted with absolute alcohol and used for the study. The oral hypoglycaemic effect, glucose tolerance test and antidiabetic activity of the *Mangifera indica* kernel seeds extracts were studied at 100 and 200 mg/kg b.wt. The antidiabetic potential of *Mangifera indica* leaves and kernel seeds extract were compared with tolbutamide 500 mg/kg b.wt.

Result: The alcoholic extract of *Mangifera indica* leaves and kernel seeds at 200 mg/kg showed significant ($p < 0.01$) hypoglycaemic effect in the fasted normal rats after 3 h of drug administration, when compared with normal group. The *Mangifera indica* leaves and kernel seeds extracts were significantly increased insulin level at the dose level of 100, 200 mg/kg in aloxone induced diabetic rats.

Conclusion: The alcoholic extract of *Mangifera indica* leaves and kernel seeds having significant antidabatic effect against aloxone induced diabetes in Wistar rats and its stimulating insulin production in pancreas of Wistar rats.

INTRODUCTION

Diabetes mellitus is the most common endocrine disorder that affects more than 100 million people worldwide. Diabetes is a heterogeneous primary disorder of carbohydrate metabolism and it is affected around 171 million people worldwide in 2000 and it may be increase to atleast 366 million by 2030.[1, 2, 3] Major limitations of the oral antidiabetic agent are their side effects and cost of the therapy. The high cost of modern treatment of diabetes indicates a great need for the development of alternative strategies for prevention and treatment of diabetes. Origin and natural products play an important role in drug development programs in the pharmaceutical industry and plants are the basic source of knowledge of modern medicine.[4]

There have been many studies on hypoglycaemic plants and a great variety of compounds have been isolated, but the main bottleneck is the further development of such 'leads' into clinically useful medicines and especially phytomedicines are adequate nutritional supplements, which would be of direct benefits to patients. In this context, it is important to remember that the modern drug metformin is a derivate of an active natural product from the plant *Galega officinalis*.[5] The experimental antidiabetic, antifungal, immunomodulatory and antioxidant activity of crude extracts of *Mangifera indica* (Anacardiaceae) plant parts were reported in earlier studies.[6, 7, 8, 9] There is no study on antidiabetic effect of kernel seeds; hence the present study was planted to determine the possible antidiabetic effect of *Mangifera indica* kernel seeds and its comparison with the leaves.

MATERIAL AND METHODS

Mangifera indica (Anacardiaceae) is a tree and distributed in rural and semi urban parts of the India. The taxonomically identified *Mangifera indica* leaves and fruits were collected in the months of Mach- May (2009) in the regions of Madurai district and certified by Taxonomist, American college, Madurai. The voucher specimen of the plant was deposited in the Department of Pharmacology, Ultra College of Pharmacy for further reference.

Preparation of Mangifera indica kernel seeds extract (MKE): The shadow, air dried kernel seeds of *Mangifera indica* were powdered and used for the extraction. The powdered kernel seeds were extracted with absolute ethanol by cold maceration process for 48 h. The extract was filtered, concentrated and dried at 60^0C.[10]

Preparation of Mangifera indica leaves extract (MLE): The shadow, air dried leaves of *Mangifera indica* were powdered and used for the extraction. The leaf power was extracted with absolute ethanol using soxhlet apparatus for 6 h. The residue was concentrated and dried at 60^0C.

Preparation of dosage forms: The MKE and MLE were suspended with minimum volume of 1% w/v Tween 80 and volume was adjusted with distilled water. The tablet of tolbutamide (Lotus Pharma Ltd.) was triturated and equivalent weighed quantity of tolbutamide (500mg/kg) was prepared with distilled water. Alloxan monohydrate (S.D. fine-Chem ltd., Mumbai) (120 mg/kg) was dissolved in sterile normal saline and used for the experiment.

Animals: Healthy, adult male Wistar rats weighing 180-200 g were used for study. The animals were housed in large and spaces polypropylene cages, maintained under standard condition (12 h Light/12 h dark cycle, 25 ^{0}C and 30-35% humidity) and fed with standard pellet diet (M/s. Hindustan lever Ltd., Bangalore, India) and water *ad libitum*. The study was approved by institutional

animal ethics committee of Ultra College of Pharmacy, Madurai. All the animals experimental procedure were carried out as per CPCSEA guideline. (CPCSEA No. 890/ac/05/ CPCSEA)

Effect of MKE and MLE on blood glucose level in normal fasted rats: Overnight fasted rats were divided into six groups of six rats each. Group I animals were received drug vehicle (distilled water) and group II animals were received tolbutamide (500 mg/kg) by oral route. A dose 100 mg/kg and 200 mg/kg of alcoholic extract of MKE (to groups III and IV animals) and MLE (to groups V and VI animals) were suspended in drug vehicle and administered orally. The blood sample was collected in tail vein and blood glucose levels were measured by using glucometer just prior to the experiment (pre-study) and 1, 2 and 3 h after dosing.[4]

Effect of MKE and MLE on Oral Glucose Tolerance Test: The oral glucose tolerance test was performed in overnight fasted rats. Thirty six rats were divided into six groups and each of six animals as follows,

Group I : Normal control
Group II : Tolbutamide 500 mg/kg
Group III : MKE 100 mg/kg
Group IV : MKE 200 mg/kg
Group V : MLE 100 mg/kg
Group VI : MLE 200 mg/kg

All the animals were received glucose (3 g/kg p.o.) 30 min after dosing. Blood glucose level was measured by using glucometer at 0 (pre-study) and 30, 60, 90, 120 min after drug administration. The micro volume of blood samples were collected from tail vein for glucose estimation.[4]

Effect of MKE and MLE on alloxan induced diabetic rats: The hyperglecemia was induced using single intraperitoneal injection of 120mg/kg alloxan monohydrate in 18 h fasted rats. After 1 h alloxan administration the animals were fed with standard rat pellet diet and water *ad libitum.* The blood glucose level was monitored after 72 h of alloxanization. The blood sample was collected by tail vein and glucose level was estimated using glucometer. The rats having blood glucose level above 150 mg/dl were selected for the study. The hyperglecemic animals were randomly divided in to seven groups and each group contains six animals as follows,

Group I : Normal control
Group II : Diabetic control
Group III : Tolbutamide 500 mg/kg
Group IV : KE 100 mg/kg
Group V : MKE 200 mg/kg
Group VI : LE 100 mg/kg
Group VII : LE 200 mg/kg

The drug vehicle, standard drug and test substance were administered once daily, per orally for the period of 21 days. All the drug administration procedure was carried out between 8-9:30 am of the day. The blood glucose levels were estimated on 0 (pre-study) and 7, 14, 21 day of the study. The rats were restrained in rat restrainer and blood samples were collected from the tail vein by making a small incision on the tail tip and 0.5-1.0 ml of the blood was collected for estimation of blood glucose, hemoglobin and serum insulin. The Blood glucose level was determined by using glucometer and blood glucose test strips (CONTOUR^{TM}TS). Hemoglobin leads to formation of glycohemoglobin throughout the circulatory life of RBC by addition of glucose to N-terminal of hemoglobin beta chain. This process is nonenzymatic, reflects the average exposure of hemoglobin to glucose over an extended period.[11, 12] The insulin concentration was calculated by Enzyme linked immuno sorbent assay (ELISA).[1]

Statistical analysis: The mean ± SEM values were calculated for each group. Significant difference between groups was

determined using analysis of variance (ANOVA) followed by Dunnett's t test. P<0.05 was considered as significant.

RESULTS

The yield of MKE and MLE were 3.55 % w/w and 5.34 % w/w respectively. The MKE and MLE were obtained in dark brown colour and green colour respectively. As the extract was observed deliquescent it was stored in air tight container in a dry place until it was used for further study.

Effect MKE and MLE on blood glucose level in normal fasted rats: The MKE at 200 mg/kg showed significant (p<0.01) hypoglycaemic effect in the fasted normal rats after 3 h of oral administration, when compared with normal group. The MKE at 100 mg/kg and MLE at 100 mg/kg and 200 mg/kg failed to show any significant reduction in blood glucose level within the period of study. The standard drug tolbutamide (500 mg/kg) p.o. also produced significant (p< 0.01) hypoglycaemic effect in fasted normal rats after 2 h. Results are tabulated in table No.1.

Table No. 1
Effect of MKE and MLE on normal fasted rats

Group	Treatment	Blood glucose level in mg/dl			
		0 h	1 h	2 h	3 h
I	Vehicle	82.46 ± 1.60	81.7 ± 1.54	80.47 ± 1.57	79.4 ± 1.48
II	Tolbutamide 500mg/kg	83.57 ± 1.14	77.93 ± 0.49	73.76 ± 0.55	69.97 ± 1.02**
III	MKE 100 mg/kg	81.13 ± 1.35	80.1 ± 1.40	78.6 ± 1.59	77.67 ± 1.83
IV	MKE 200 mg/kg	79.03 ± 1.33	77.3 ± 1.54	74.7 ± 1.70	73.23 ± 1.62*
V	MLE 100 mg/kg	82.03 ± 1.53	81.57 ± 1.45	80.57 ± 1.30	79.87 ± 1.16
VI	MLE 200 mg/kg	81.33 ± 1.09	80.3 ± 1.71	78.0 ± 1.80	77.0 ± 1.67

*(Values are mean ± SEM of sex animals); *p<0.05, **p<0.01 as compared to control.*

Effect of MKE and MLE on Oral Glucose Tolerance Test: The MKE showed significant glucose reduction at 200 mg/kg (p< 0.01) dose level after 60 min of MKE administration, when compared to control group. The standard drug tolbutamide (500 mg/kg) also produced significant (p< 0.01) reduction in blood glucose level from 30 min onwards. The MKE at 100 mg/kg produced significant reduction in blood glucose level after 120 min but MLE at 100 mg/kg failed to produce any significant reduction in blood glucose level (Table No.2).

Table No. 2
Effect of MKE and MLE on oral glucose tolerance test in normal rats

Group	Treatment	Blood glucose level in mg/dl				
		0 min	30 min	60 min	90 min	120 min
I	Vehicle	95.16 ± 1.22	149.2 ± 1.1	138.03 ± 2.52	129.47 ± 1.16	121.3 ± 1.07
II	Tolbutamide 500mg/kg	91.33 ± 0.93	140.93 ± 1.77*	122.17 ± 1.8**	97.6 ± 1.46**	87 ± 1.28**
III	MKE 100 mg/kg	94.3 ±	144.7 ±	139.97 ±	122.3 ±	108.87 ±

		1.66	1.74	1.87	0.98*	2.21**
IV	MKE 200 mg/kg	94.23 ± 2.53	143.1 ± 2.17	127.37 ± 2.60*	109.4 ± 1.67**	99.23 ± 1.53**
V	MLE 100 mg/kg	92.06 ± 1.45	147.63 ± 1.47	135.77 ± 1.87	127.4 ± 1.88	116.07 ± 0.82
VI	MLE 200 mg/kg	91.57 ± 1.58	144.27 ± 1.42	130.23 ± 1.94	118.27 ± 1.13**	106.43 ± 0.63**

*(Values are mean ± SEM of sex animals); *p<0.05, **p<0.01 as compared to control.*

Effect of MKE and MLE on insulin level in alloxan induced diabetic rats: The oral administered MKE (100, 200 mg/kg) and MLE (100, 200 mg/kg) showed increased insulin level in a dose dependent manner after 21 days treatment period, compared with diabetic control group. The MKE (100, 200 mg/kg p.o.) and MLE (100, 200 mg/kg p.o.) produced sustained and significant (p< 0.01) antidiabetic activity against alloxan induced diabetics and significantly increased insulin level. The standard drug tolbutamide (500 mg/kg p.o) elevated the insulin level to the normal i.e. 7.53 mIu, while MKE and MLE (both at 200 mg/kg each) elevated the insulin level to 6.87 mIu and 6.7 mIu respectively, on 21st day. The dose dependant increase was observed, as MKE and MLE at 100 mg/kg each elevated serum insulin level to 4.6 mIu and 4.2 mIu on 21st day (Table No.3). The standard drug tolbutamide (500 mg/kg p.o) and MKE (at 200 mg/kg p.o.) has been shown more than 50% of reduction while MLE (at 200 mg/kg p.o) has been shown more than 45% in blood glucose level while MKE (at 100 mg/kg p.o) has been shown near about 30% reduction and MLE (at 100 mg/kg p.o) has been shown 14% reduction in blood glucose level (Table No. 3, 4).

Table No. 3

Effect of MKE and MLE on serum Insulin level in alloxan induced diabetic rats

Group	Treatment	Serum insulin level in mIu/ml			
		0th day	7th day	14th day	21st day
I	Vehicle	7.33 ± 0.26	7.8 ± 0.26	7.56 ± 0.14	7.6 ± .23
II	Diabetic control	0.2 ± 0.06	0.23 ± 0.03	0.23 ± 0.03	0.33 ± 0.03
III	Tolbutamide 500mg/kg	0.13 ± 0.17	1.23 ± 0.09**	4.63 ± 0.22**	7.53 ± 0.18**
IV	MKE 100 mg/kg	0.16 ± 0.07	0.56 ± 0.09	1.26 ± 0.09**	4.6 ± 0.21**
V	MKE 200 mg/kg	0.13 ± 0.03	0.93 ± 0.09**	3.56 ± 0.18**	6.87 ± 0.14**
VI	MLE 100 mg/kg	0.16 ± 0.03	0.53 ± 0.09	1.03 ± 0.09**	4.2 ± 0.17**
VII	MLE 200 mg/kg	0.16 ± 0.03	0.96 ± 0.12**	3.03 ± 0.09**	6.7 ± 0.25**

*(Values are mean ± SEM of sex animals); *p<0.05, **p<0.01 as compared to control.*

Table No. 4

Effect of MKE and MLE on blood glucose level in alloxan induced diabetic rats

Group	Treatment	Blood glucose level in mg/dl			
		0th day	7th day	14th day	21st day
I	Vehicle	79.6 ± 1.44	79.16 ± 1.3	79.8 ± 1.69	80.4 ± 2.12

II	Diabetic control	254.1 ± 3.57	245.17 ± 2.38	239.2 ± 0.83	231.6 ± 1.38
III	Tolbutamide 500mg/kg	253.23 ± 3.15	162.03 ± 1.43**	110.13 ± 2.92**	93.93 ± 2.43**
IV	MKE 100 mg/kg	255.57 ± 2.77	233.6 ± 3.38	195.93 ± 2.94*	161± 1.97**
V	MKE 200 mg/kg	254.63 ± 3.76	170.2 ± 1.04**	130.33 ± 1.78**	112.2 ± 2.26**
VI	MLE 100 mg/kg	257.93 ± 2.77	236.73 ± 3.46	227.73 ± 2.54*	197.4 ± 2.46**
VII	MLE 200 mg/kg	257.83 ± 3.20	182.9 ± 2.11**	141.47 ± 1.48**	126.4 ± 1.91**

*(Values are mean ± SEM of sex animals); *p<0.05, **p<0.01 as compared to control.*

Effect of MKE and MLE on hemoglobin in alloxan induced diabetic rats: End of the study MKE (100, 200 mg/kg) and MLE (100,

200 mg/kg) treated animals showed dose dependent increase in hemoglobin level, compared with diabetic control animals. The MKE (100, 200 mg/kg p.o.) and MLE (100, 200 mg/kg) produced sustained significant (p< 0.01) antidiabetic activity during prolonged treatment indicated by reduction in glucose levels and elevation of hemoglobin level throughout treatment for 21 days. The standard drug tolbutamide (500 mg/kg p.o) elevated the hemoglobin level by 75.22 %, while MKE and MLE (both at 200 mg/kg each) elevated the hemoglobin level by 66.05 % and 55.04 % respectively, on 21st day. The dose dependant increase was observed, as MKE and MLE at 100 mg/kg each elevated haemoglobin level by 38.40 % and 12.18 % respectively, on 21st day. According to previous literatures, the elevated blood glucose level converts haemoglobin to glycosylated haemoglobin. Hence haemoglobin level in blood decreased through 21 days of observations of diabetic control group. But this needs further investigation of glycosylated haemoglobin (Table No.5).

Effect of MKE and MLE on body weight in alloxan induced diabetic rats: There was a significant weight loss in the vehicle treated diabetic rats, whereas animals treated with MKE and MLE at the doses of 200 mg/kg p.o. showed the significant increase in weight 14th day onwards, indicating that the MKE and MLE had beneficial effects in preventing loss of body weight of diabetic rats (Table No.6).

Table No. 5

Effect of MKE and MLE on hemoglobin in alloxan induced diabetic rats

Group	Treatment	Hemoglobin level in g %			
		0th day	7th day	14th day	21st day
I	Vehicle	13.93 ± 0.15	13.83 ± 0.19	14.13 ± 0.55	14.07 ± 0.44
II	Diabetic control	9.53 ± 0.18	8.5 ± 0.15	7.56 ± 0.20	7.63 ± 0.15
III	Tolbutamide 500mg/kg	9.53 ± 0.19	11.43 ± 0.12**	12.63 ± 0.12**	13.37 ± 0.15**
IV	MKE 100 mg/kg	9.63 ± 0.18	9.16 ± 0.08	10.1 ± 0.11**	10.56 ± 0.20**
V	MKE 200 mg/kg	9.56 ± 0.23	11 ± 0.05**	11.86 ± 0.14**	12.67 ± 0.15**

VI	MLE 100 mg/kg	9.73 ± 0.09	9.03 ± 0.06	8.33 ± 0.16*	8.56 ± 0.09*
VII	MLE 200 mg/kg	9.5 ± 0.25	10.53 ± 0.18**	11.3 ± 0.15**	11.83 ± 0.18**

*(Values are mean ± SEM of sex animals); *p<0.05, **p<0.01 as compared to control.*

Table No. 6
Effect of MKE and MLE on body weight in alloxan induced diabetic rats

Group	Treatment	Body weight (g)			
		0th day	7th day	14th day	21th day
I	Vehicle	165.66 ± 4.91	166 ± 4.04	165.66 ± 2.96	164.66 ± 1.86
II	Diabetic control	162 ± 1.73	154.66 ± 1.45	147.66 ± 1.67	145 ± 2.08
III	Tolbutamide (500 mg/kg)	160.33 ± 3.38	166 ± 3.06*	169.67 ± 2.73**	172.33 ± 2.85**
IV	MKE-100	158.33 ± 2.18	161 ± 1.53	160.66 ± 0.88**	161.33 ± 1.33**
V	MKE-200	157.66 ± 2.33	165.33 ± 1.33	168 ± 1.53**	170.67 ± 1.20**
VI	MLE-100	163.33 ± 3.93	165.33 ± 3.93	165.67 ± 4.18**	165.67 ± 4.26**
VII	MLE-200	162.66 ± 2.33	166 ± 1.53*	168.67 ± 1.45**	170.67 ± 0.67**

*(Values are mean ± SEM of sex animals); *p<0.05, **p<0.01 as compared to control.*

DISCUSSION

The hypoglycemic effects of alcoholic extracts of *Mangifera indica* kernel seeds extract at 200 mg/kg detected in present study is a direct evidence of the stimulation of insulin secretion. The activity was comparable to that of the standard drug which reinforces the insulin secretogogoue/ pancreotrophic action of the extract.[2, 4] Tolbutamide (sulfonyl urea) causes hypoglycemia by stimulating insulin release from pancreatic β-cells.[13]

The oral glucose tolerance test showed that the MKE gave significant lower blood glucose level at the end of 60 min and the same with MLE at the end of 90 min after glucose loaded and even lower level at end of 120 min. The MKE and MLE enhanced glucose utilization, so the blood glucose level was significantly decreased in glucose loaded rats.

The ethanolic extract of kernel seeds and leaves of *Magifera indica* showed significant hypoglycaemic effect in hyperglycaemic rats and antidiabetic activity in alloxan induced diabetic model. Alloxan 120 mg/kg i.p. was used to induce diabetics in rats and it was partially destroy the pancreatic β-cells. Alloxan, a β-cytotoxin, induces "chemical diabetes" in a wide variety of animal species including rats by damaging the insulin-secreting β-cells. In alloxan induced diabetic rats the hyperglycemia was permanent and it was varied from 230 mg/dl to 260 mg/dl.[14]

Hemoglobin leads to formation of glycohemoglobin throughout the circulatory life of RBC by addition of glucose to N-terminal of hemoglobin beta chain. This process is nonenzymatic, reflects the average exposure of hemoglobin to glucose over an extended period.[11] Treatment with ethanolic extract of *M. indica* (100 mg/kg, 200 mg/kg) for both MKE and MLE for the period of 21 days showed the significant increase in the hemoglobin level in diabetic rats. Maximum

elevation of hemoglobin level occurred at dose of 200 mg/kg of MKE, p.o. The hemoglobin level was decreased in diabetic rats that may increase the formation of glycosylated hemoglobin. Glycosylated hemoglobin was found to be increased in diabetic mellitus and the amount of increase is directly proportional to that of fasting blood glucose level.[1] The significant increase in haemoglobin indicated that the efficiency of MKE and MLE in glycemic control. But further investigation of glycosylated hemoglobin is needed in support of antidiabetic activity based on investigation of hemoglobin.

Continuous treatment with ethanolic extract of *M. indica* (100 mg/kg, 200 mg/kg) for both MKE and MLE for the period of 21 days showed the significant increase in the serum insulin level in diabetic rats. The decrease in level of blood glucose and increased level of insulin were observed in our present study, which indicates that MKE and MLE stimulates insulin secretion from the remnant β-cells or regenerated β-cells. There was a significant weight loss in the vehicle treated diabetic rats, whereas treatment with ethanolic extract of kernel seeds and leaves of *M. indica* at the doses of 200 mg/kg showed the improvement in their body weights, indicating that the ethanolic extract had beneficial effects in preventing loss of body weight of diabetic rats.[15]

CONCLUSION

The ethanolic extract of leaves and kernel seeds of *Magifera indica* to alloxan treated diabetic rats is a restoration in blood glucose level and insulin. Moreover it showed elevation in hemoglobin and body weight. The present study indicates that a significant antidiabetic effect of MKE and MLE potentiate the β-cells of pancreas. So, the leaves and kernel seeds possess the significant antidiabetic activity and acts by stimulating the insulin production from the pancreas and it supports to control the diabetes and its complications.

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