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Original Research Article

Evaluation of antioxidant potential of *Emblica officinalis* and *Murraya koenigii* and their role in modulation of cognitive function in diabetic rats

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ABSTRACT

Background: Hyperglycaemia and hyperlipidaemia seen in diabetes mellitus result in oxidative stress and pose significant risk of cognitive decline that may lead to Alzheimer's disease. Approved anti-diabetic drugs have so far failed to demonstrate anti-oxidant and anti-hyperlipidemic activity, apart from saroglitazar. Therefore, this study was done to find a suitable anti-diabetic drug that possesses anti-hyperglycaemic, anti-oxidant and anti-hyperlipidemic activities and can reverse cognitive decline.
Methods: *Emblica officinalis* (250 mg/kg, p.o. and 500 mg/kg, p.o.) and *Murraya koenigii* (250 mg/kg, p.o. and 500 mg/kg, p.o.) were chosen to study these activities in Wistar rats. Diabetes was induced by single intraperitoneal injection of streptozotocin [STZ] (50 mg/kg). Fasting blood glucose levels and lipid profile were measured on day 1 and day 30 of the experiment. Cognitive function was assessed by measuring transfer latency (TL) on elevated plus maze, step-down latency (SDL) on passive avoidance apparatus and retention latency (RL) and quadrant time (QT) in Morris water maze. Oxidative stress was assessed at end of study by measuring brain MDA and GSH levels. Cholinergic marker of cognition, AChE was measured in brain at end of study.
Results: Both *E. officinalis* and *M. koenigii* showed dose dependent anti-hyperglycemic, anti-hyperlipidemic and anti-oxidant effects in diabetic rats with 500 mg/kg dose showing significantly higher effect. Both 250 mg/kg and 500 mg/kg dose of *E. officinalis* and *M. koenigii* partially reversed cognitive decline in diabetic rats by day 30.
Conclusions: 500 mg/kg p.o. dose of *E. officinalis* or *M. koenigii* has potential to reverse cognitive decline in diabetic patients.

Keywords: *Emblica officinalis*, *Murraya koenigii*, Oxidative stress, Cognitive decline, Diabetes

INTRODUCTION

Diabetes mellitus (DM), a complex syndrome is characterized primarily by imbalance in blood glucose homeostasis leading to hyperglycaemia (high blood sugar) and a series of secondary complications caused by an absolute or relative lack of insulin. Diabetes Mellitus is one of the most common endocrine disorders affecting almost 6% of the world's population. The number of diabetic patients will reach 300 million in 2025 (International Diabetic Federation, 2001).[1]

Despite progress in the management of diabetes mellitus by synthetic drugs most of these drugs have side effects in long run and drug resistance is also noted.[2] So, the search for improved and safe natural antidiabetic agents is ongoing and World Health Organization has also recommended the development of herbal medicine in this concern.[3] There is association of diabetes mellitus with cognitive dysfunction which is paralleled by neurophysiological and structural changes in CNS.[4]

Emblica officinalis (Family - Euphorbiaceae), popularly known as Amla is one of the most celebrated herbs in

Indian traditional medicine system, Ayurveda. Amla is highly nutritious and is an important dietary source of Vitamin C, minerals and amino acids. The fruit contains considerably higher concentration of most minerals and amino acids and has been reported to possess expectorant, purgative, spasmolytic, antibacterial, hypoglycemic, hepatoprotective and hypolipidemic activity.[5-9]

Murraya koenigii (syn. *Bergera koenigii*, *Chalcas koenigii*) is a tropical to sub-tropical plant belonging to family *Rutaceae*, which is native to India. It produces the leaves known as Curry leaves or Sweet Neem leaves. The hypoglycaemic effect of curry leaves has been studied in animal models. It has also been shown to possess antioxidant properties.[10]

As described above, both *E. officinalis* and *M. koenigii* possess antioxidant and hypoglycaemic properties. Considering the role of oxidative stress and the resultant cognitive impairment in diabetes, present study was done for evaluation of cognitive impairment and antioxidant potential of *E. officinalis* and *M. koenigii* in streptozotocin-induced diabetic rats.

METHODS

Animals

Wistar rats of either sex weighing between 150 g and 200 g were taken for the study. The rats were randomly distributed into different groups and were placed in polyvinyl cages. Each group contained ten rats, and the rats were numbered. The animals were housed under standard laboratory conditions and were fed with standard pelleted laboratory diet and water ad libitum. On the day of experiment rats were fasted overnight to avoid influence of food on drug absorption though water was given. Animals were maintained on a 12 hour natural light- dark cycle; temperature 22±1°C and 50±2% relative humidity, with free access to food and water. The rats were acclimatized to laboratory conditions prior to experimentation.

Ethics committee approval was taken from the Institutional Animal Ethics Committee and the care of animals was done as per "CPCSEA Guidelines" for laboratory animal facilities.

Drugs

Distilled water, physiological saline, commercially available extract of fruit of *E. officinalis*, commercially available extract of leaves of *M. koenigii*, gliclazide, vitamin E, melatonin, atorvastatin.

Chemicals

Streptozotocin, other laboratory chemicals as will be required for estimation of biochemical parameters.

Experimental design

Induction of diabetes

Diabetes was induced by administering single intraperitoneal (i.p) injection of streptozotocin (50 mg/kg) dissolved in citrate buffer of pH 4.5 in overnight fasted rats. The rats which had blood glucose levels >200 mg/dl (fasting glycaemia) were considered as diabetic rats.

Measurement of different parameters

Blood glucose and serum lipid profile

Fasting blood samples were collected from the retro-orbital plexus of the eye of rat and blood sugar was estimated by using glucose oxidase kit.

Fasting blood glucose levels were estimated till blood glucose levels stabilized i.e., BG >200 mg/dl and drug treatment was started from next day of blood glucose stabilization (which was taken as day 1) and continued for 30 days.

Blood samples were collected one day before the induction of diabetes and on 15th and 30th day after the stabilization of blood glucose levels for estimation of blood sugar and lipid profile (TC, LDL-c, HDL-c and TG).

Cognitive assessment

Behavioural tests to assess learning and memory were performed one day before the induction of diabetes and on 15th and 30th day after the stabilization of blood glucose levels.

Oxidative stress

At the end of treatment, rats were sacrificed under ether anaesthesia and brain was taken out. Brain malondialdehyde, reduced glutathione and acetylcholinesterase levels were estimated.

In the experiment the rats were divided into following groups (n=10) to study the antidiabetic and antioxidant effects of different doses of *E. officinalis* and *M. koenigii* and their role in modulation of cognitive dysfunction.

Assessment of anti-hyperglycaemic and hypolipidemic activity

- Group I Control (Distilled water): Vehicle of *E. officinalis*/ *M. koenigii* per oral (p.o).
- Group II Diabetic control: Streptozotocin (50 mg/kg, i.p).
- Group III Diabetic rats: *E. officinalis* per se; Dose I (250 mg/kg, p.o).

- Group IV Diabetic rats: *E. officinalis* per se; Dose II (500 mg/kg, p.o).
- Group V Diabetic rats: *M. koenigii* per se; Dose I (250 mg/kg, p.o).
- Group VI Diabetic rats: *M. koenigii* per se; Dose II (500 mg/kg, p.o).
- Group VII Diabetic rats: Gliclazide (20 mg/kg, p.o).
- Group VIII Diabetic rats: Atorvastatin (10 mg/kg, p.o).

Assessment of antioxidant activity and cognitive function

Additional two groups of diabetic rats were taken for assessment of antioxidant activity and cognitive function as given below.

- Vitamin E+STZ: 100 mg/kg, p.o./daily+50 mg/kg i.p.
- Melatonin+STZ: Melatonin 50 mg/kg, p.o./daily+50 mg/kg i.p.

Subsequently these groups were compared with the drug treated groups of *E. officinalis* and *M. koenigii*.

Assessment of oxidative stress

Estimation of malondialdehyde

Malondialdehyde (MDA) (indicator of lipid peroxidation) was estimated as per procedure described by Okhawa et al.[11]

Estimation of reduced glutathione

Reduced glutathione (GSH) was estimated by the method as described by Ellman.[12]

Estimation of brain acetylcholinesterase activity

The rate of formation of thiocholine from acetylthiocholine iodide in the presence of tissue cholinesterase (AChE) is measured by first treating with DTNB and then measuring the optical density (OD) of yellow coloured compound formed during the reaction at 412 nm, every minute for a period of three minutes as per Ellman et al.[13]

Assessment of cognition

Transfer latency on elevated plus maze

Transfer latency (TL) was assessed through elevated plus maze test. TL is the time in which the animal moves from open arm to closed arm. The animal which does not enter a closed arm within 90 sec, were not included in the experiment. To become acquainted with the maze the animals were allowed to explore the maze for 20 s after reaching the closed arm and were then returned to their home cage. Retention was examined 24 h after the first day trial.

Step down latency in continuous avoidance apparatus

The rat was placed on the shock free zone (SFZ) and on stepping- down was given electric shock (20V) through the grid floor. The animal was returned to its cage and after 24 h, the procedure was repeated and the time taken for the rat to step down was measured. This is known as step down latency (SDL). A prolongation of SDL was used as parameter of learning.

The animal groups were evaluated for TL and SDL one day before the induction of diabetes and on 14^{th} day and 29^{th} day after stabilization of blood glucose levels.

Morris water maze (Spatial navigation task)

The animal groups were evaluated for spatial navigation task on Morris water-maze one day before the induction of diabetes and on 15^{th} day and 30^{th} day after stabilization of blood glucose levels.

Statistical analysis

Results were analysed by one way Anova followed by post hoc Turkey's test for inter group comparison. Value of $p<0.05$ was taken as significant and value of $p<0.001$ was taken as highly significant.

RESULTS

Effect of various drugs (x30 days) on blood glucose levels

There was significant ($p<0.001$) increase in blood glucose levels in Diabetic control (II) group compared to non-diabetic control (I) group. At day '30' *E. officinalis* dose 250 mg/kg (III) group, Group IV, Group V, Group VI and Group VII showed "significant decrease" ($p<0.001$) in blood glucose levels when compared to Group II. Melatonin group (50 mg/kg), Vitamin E group (100 mg/kg) and group VIII did not show any significant change in blood glucose levels when compared to diabetic control group (Table 1).

Effect of various drugs (x30 days) on serum total cholesterol levels

Group II (diabetic control) showed "significant increase" ($p<0.001$) in total cholesterol levels at day '30' as compared to Group I. In comparison with Group II; the groups which showed "significant decrease" in total cholesterol levels were Group III ($p<0.05$), Group IV ($p<0.01$), Group V ($p<0.05$), Group VI ($p<0.001$) and Group VIII ($p<0.001$). Melatonin group, Vitamin E group and Group VII did not show any significant change in total cholesterol level when compared to Group II (Table 2).

Table 1: Effect of various drugs (x30 days) on blood glucose in STZ induced-diabetic rats (n=10).

Group	Dose and route (mg/kg, p.o.)	Blood glucose (mg/dl) Mean±SEM	
		Day 0	Day 30
Distilled water (non-diabetic Control)	Vehicle of *E. officinalis* and *M. koenigii*	74±4.61	78.7±1.28
Streptozotocin (diabetic control)	50, i.p.	291±5.22[a]	298±4.36[a]
***E. officinalis* Dose I+STZ**	250	278.2±3.27[a]	166.7±2.44[a,b]
***E. officinalis* Dose II+STZ**	500	287.5±2.39[a]	161.4±3.43[a,b]
***M. koenigii* Dose I+STZ**	250	281±3.23[a]	188±2.67[a,b]
***M. koenigii* Dose II+STZ**	500	276±1.41[a,c]	152±1.22[a,b]
Melatonin+STZ	50	272.2±2.42[a]	289.6±2.24[a]
Vitamin E+STZ	100	275.7±3.41[a]	294.1±1.43[a]
Gliclazide+STZ	20	281.7±2.43[a]	164.9±1.62[a,b]
Atorvastatin+STZ	10	288.2±1.41[a]	290.6±2.11[a]

[a]p<0.001 as compared with non- diabetic control (distilled water) group; [b]p<0.001 as compared with diabetic control (STZ) group; [c]p<0.05 as compared with diabetic control (STZ) group; One-way ANOVA followed by post-hoc Tukey's test.

Table 2: Effect of various drugs (x30 days) on serum total cholesterol (TC), Triglycerides (TG), LDL-c and HDL-c levels in STZ induced-diabetic rats (n=10).

Group	Dose and route (mg/kg, p.o.)	Serum total cholesterol, triglycerides, LDL-c and HDL-c (mg/dl) Mean±SEM			
		Day 30 (TC)	Day 30 (TG)	Day 30 (LDL-c)	Day 30 (HDL-c)
Distilled water (non-diabetic control)	Vehicle of *E. officinalis* and *M. koenigii*	135.2±3.74	120.6±12.76	65.4±3.74	34±1.98
Streptozotocin (diabetic control)	50, i.p.	188.3±10.7[a]	175.2±5.91[d]	84.9±7.98	23.6±3.76
***E. officinalis* Dose I+STZ**	250	155.4±6.87[d]	89.3±12.62[a]	51.9±8.29[d]	34.9±2.27
***E. officinalis* Dose II+STZ**	500	145.1±3.49[c]	84±3.85[a]	47.4±9.32[c]	41.1±4.08[c]
***M. koenigii* Dose I+STZ**	250	155.6±2.43[d]	103.9±8.83[a]	56.7±6.13	40.2±4.72[d]
***M. koenigii* Dose II+STZ**	500	140.7±7.34[b]	81.1±11.08[a]	51.2±4.67[d]	44.2±2.12[a]
Melatonin+STZ	50	186.2±9.29[a]	131.7±14.28	78.7±4.65	30.6±3.76
Vitamin E+STZ	100	180.1±5.43[a]	138.2±16.87	71.5±7.94	32.5±1.87
Gliclazide+STZ	20	165.8±9.93	124.5±12.8	70.1±3.63	33.6±3.22
Atorvastatin+STZ	10	130.3±1.87[b]	80.3±5.09[a]	48.3±4.47 [d]	38.2±2.65[d]

[a]p<0.001 as compared with non-diabetic control (distilled water) group; [b]p<0.001 as compared with diabetic control (STZ) group. [c]p<0.01 as compared with diabetic control (STZ) group; [d]p<0.05 as compared with diabetic control (STZ) group; One-way ANOVA followed by post-hoc Tukey's test

Effect of various drugs (x30 days) on serum total triglyceride levels

At day '15', "significant increase" in total triglyceride was seen in Group II (p<0.001) as compared to Group I. In comparison with Group II; the groups which showed "significant decrease" in total triglyceride levels at day '15' were *E. officinalis* dose 500 mg/kg (IV) group (p<0.001) and Group VIII (p<0.001).

At day '30', "significant increase" in total triglyceride was seen in Group II (p<0.05) as compared to Group I. In contrast to this, "significant decrease" in total triglyceride

levels were seen in Group III, IV and VIII (p<0.01*) M. koenigii* dose 250 mg/kg (V) group and *M. koenigii* dose 500 mg/kg (VI) group showed highest reduction in triglyceride levels (p<0.001) (Table 2).

Effect of various drugs (x30 days) on serum total LDL-cholesterol levels

At day '0' and '15' none of the studied compounds produced significant change in serum LDL-cholesterol levels when compared to Group II. At day '30'; the groups which showed "significant decrease" in serum LDL-cholesterol levels in comparison with Group II were Group III (p<0.05), Group IV (p<0.01), Group VI (p<0.05) and Group VIII (p<0.05) (Table 2).

Effect of various drugs (x30 days) on serum HDL-cholesterol levels

At day '30'; the groups which showed "significant increase**"** in serum HDL-cholesterol levels in comparison with Group II were Group IV (p<0.01), Group V (p<0.05), Group VI (p<0.001) and Group VIII (p<0.05). (Table 2)

Effect of various drugs (x30 days) on behavioral parameters of learning and cognition

Acquisition of transfer latency (TL) in elevated plus-maze

No significant difference in TL was seen in any group at day '0' and day '14' as compared to Group I.

Table 3: Effect of various drugs (x30 days) on acquisition of transfer latency in STZ induced-diabetic rats (n=10).

Group	Dose and route (mg/kg, p.o.)	Transfer latency (seconds) Mean±SEM		
		Day 0	Day 14	Day 29
Distilled water (non-diabetic control)	Vehicle of *E. officinalis* and *M. koenigii*	68.7±5.54	59.4±7.23	31.5±3.27
Streptozotocin (diabetic control)	50, i.p.	86.6±6.78	74.7±2.63	64.7±4.07[a]
***E. officinalis* Dose I+STZ**	250	81.4±4.67	59.9±1.27	51.6±5.28
***E. officinalis* Dose II+STZ**	500	74.3±3.65	53±5.76	38.4±2.87[d]
***M. koenigii* Dose I+STZ**	250	84.8±3.65	62.1±3.88	57.3±6.12[b]
***M. koenigii* Dose II+STZ**	500	76.4±2.05	58.7±2.45	43.7±4.41
Melatonin+STZ	50	69.4±4.76	58.6±3.76	46±6.55
Vitamin E+STZ	100	74.6±5.21	67.6±4.67	54.6±5.41[c]
Gliclazide+STZ	20	76.5±2.81	65.9±2.56	53.4±2.78
Atorvastatin+STZ	10	78.6±5.09	61.7±2.77	56.7±4.38[b]

[a]p<0.001 as compared with non-diabetic control (distilled water) group; [b]p<0.01 as compared with non-diabetic control (distilled water) group; [c]p<0.05 as compared with non-diabetic control (distilled water) group; [d]p<0.01 as compared with diabetic control (STZ) group. One-way ANOVA followed by post-hoc Tukey's test.

At day '29', "significant increase**"** in acquisition of TL was seen in Group II (p<0.001) as compared to Group I. In comparison to Group II; the groups which showed "significant decrease**"** in acquisition of TL were Group IV (p<0.01), Group V (p<0.01), Vitamin E group (p<0.05) and Group VIII (p<0.01) (Table 3).

Retention of transfer latency (TL) in elevated plus-maze

No significant difference was seen in any group at day '1' and day '15' as compared to Group I. At day 30, "significant increase**"** in retention of TL was seen in Group II (p<0.01) as compared to Group I.

"Significant decrease" in retention of TL was seen in Group IV (p<0.01), Group VI (p<0.01), and melatonin group (p<0.001) as compared to Group II (Table 4).

Acquisition of step-down latency (SDL) in continuous avoidance apparatus

At day '14' "significant decrease**"** in acquisition of SDL was seen in Group II (p<0.01) as compared to Group I and "significant increase**"** in acquisition of SDL was seen in Group III (p<0.01), Melatonin group (p<0.05) and Group VII (p<0.01) as compared to Group II.

Table 4: Effect of various drugs (x30 days) on retention of transfer latency in STZ induced-diabetic rats (n=10).

Group	Dose and route (mg/kg, p.o.)	Transfer latency (seconds) Mean±SEM		
		Day 1	Day 15	Day 30
Distilled water (non-diabetic control)	Vehicle of *E. officinalis* and *M. koenigii*	62±6.94	36.8±5.87	27.6±1.74
Streptozotocin (diabetic control)	50, i.p.	67.8±3.56	59.3±4.87	56.4±4.93[a]
***E. officinalis* Dose I+STZ**	250	62.4±5.57	53.7±7.11	36.9±2.94
***E. officinalis* Dose II+STZ**	500	59.5±4.93	44±5.98	30±4.08[c]
***M. koenigii* Dose I+STZ**	250	71.6±6.44	54±3.27	42±4.45
***M. koenigii* Dose II+STZ**	500	68±4.65	56±6.23	29±7.34[c]
Melatonin+STZ	50	64.6±3.68	48.8±5.22	20.7±8.19[b]
Vitamin E+STZ	100	66.3±6.23	54.3±5.79	43.5±4.74
Gliclazide+STZ	20	72.2±4.62	56.6±6.24	45.7±3.93
Atorvastatin+STZ	10	69±2.76	52±4.66	49±2.93

[a]p<0.01 as compared with non-diabetic control (distilled water) group; [b]p<0.001 as compared with diabetic control (STZ) group; [c]p<0.01 as compared with diabetic control (STZ) group; One-way ANOVA followed by post-hoc Tukey's test.

Table 5: Effect of various drugs (X 30 days) on acquisition of step-down latency in STZ induced-diabetic rats (n=10).

Group	Dose & Route (mg/kg, p.o.)	Step-down latency (seconds) Mean±SEM		
		Day 0	Day 14	Day 29
Distilled water (non-diabetic control)	Vehicle of *E. officinalis* and *M. koenigii*	182.3±12.56	235.7±7.43	274.2±3.93
Streptozotocin (diabetic control)	50, i.p.	171.7±12.63	169.7±14.98[a]	167.4±12.96[a]
***E. officinalis* Dose I+STZ**	250	182.7±11.45	237.1±5.98[d]	250.2±14.16[c]
***E. officinalis* Dose II+STZ**	500	178.5±11.64	202.5±10.43	242.8±12.43[c]
***M. koenigii* Dose I+STZ**	250	170.5±12.44	192.16±9.44	230±10.56[d]
***M. koenigii* Dose II+STZ**	500	181±14.78	204.7±15.98	244.4±12.87[c]
Melatonin+STZ	50	185.8±14.56	224.2±14.11[e]	243.2±12.43[c]
Vitamin E+STZ	100	172.4±12.82	198±11.16	214±17.45[b]
Gliclazide+STZ	20	191.5±12.97	232.5±10.66[d]	247.9±6.87[c]
Atorvastatin+STZ	10	188.5±4.87	201.6±9.54	226.3±4.23[e]

[a]p<0.01 as compared with non-diabetic control (distilled water) group; [b]p<0.05 as compared with non-diabetic control (distilled water) group; [c]p<0.001 as compared with diabetic control (STZ) group; [d]p<0.01 as compared with diabetic control (STZ) group; [e]p<0.05 as compared with diabetic control (STZ) group; One-way ANOVA followed by post-hoc Tukey's test.

At day '29', "significant decrease**"** in acquisition of SDL was seen in Group II (p<0.01) as compared to Group I. In comparison with Group II; the groups which showed "significant increase**"** in acquisition of SDL are Group III and IV (p<0.001), Group V (p<0.01), Group VI (p<0.001), Melatonin group (p<0.001), Group VII (p<0.001) and Group VIII (p<0.05).

However, Vitamin E group did not show any significant change in acquisition of TL when compared to Group II (Table 5).

Table 6: Effect of various drugs (x30 days) on retention of step-down latency in Streptozotocin (STZ) induced-diabetic rats (n=10).

Group	Dose and route (mg/kg, p.o.)	Step-down latency (Seconds) Mean±SEM		
		Day 1	Day 15	Day 30
Distilled water (Non-diabetic Control)	Vehicle of *E. officinalis* and *M. koenigii*	212.4±13.69	236.7±12.76	261±3.94
Streptozotocin (Diabetic Control)	50, i.p.	196.54±16.7	188.5±15.44[a]	190.3±18.87[a]
***E. officinalis* Dose I+STZ**	250	210.6±14.87	226.7±9.93	251.6±5.88[d]
***E. officinalis* Dose II+STZ**	500	221.7±21.66	257±10.43[b]	283.8±16.77[b]
***M. koenigii* Dose I+STZ**	250	219.6±6.65	221.7±9.66	249±13.93
***M. koenigii* Dose II+STZ**	500	208.7±12.76	216.7±7.98	252.7±19.76 [d]
Melatonin+STZ	50	212±12.46	234.6±7.66	252.8±11.63[d]
Vitamin E+STZ	100	228.4±13.34	229±12.47	264.7±9.88[c]
Gliclazide+STZ	20	213.5±13.28	235.2±5.43	264.9±7.86[c]
Atorvastatin+STZ	10	227±12.22	232.6±7.84	258.7±13.87[d]

[a]p<0.05 as compared with non-diabetic control (distilled water) group; [b]p<0.001 as compared with diabetic control (STZ) group; [c]p<0.01 as compared with diabetic control (STZ) group; [d]p<0.05 as compared with diabetic control (STZ) group; One-way ANOVA followed by post-hoc Tukey's test.

Table 7: Effect of various drugs(x30 days) on retention of spatial navigation task in STZ induced-diabetic rats (n=10).

Group	Treatment and route	Latency (seconds) Mean±SEM		
		Day 1; IAL	Day 15; RL-1	Day 30; RL-2
Distilled water (Non-diabetic Control)	Vehicle of *E. officinalis* and *M. koenigii*	40.4±7.54	38.7±5.12	48.2±3.65
Streptozotocin (Diabetic Control)	50, i.p.	49.2±2.63	72.4±3.64	95.6±4.32[a]
***E. officinalis* Dose I+STZ**	250	59.8±2.17	48.1±4.65	45.3±2.71[c]
***E. officinalis* Dose II+STZ**	500	64.5±2.71	51.4±3.97	42.9±4.17[c]
***M. koenigii* Dose I+STZ**	250	51.3±2.72	44.6±2.12	35.9±3.54[c]
***M. koenigii* Dose II+STZ**	500	63.3±2.19	59.6±6.72	43.1±3.48[c]
Melatonin+STZ	50	63.3±2.64	57±3.66	54.6±3.56[c]
Vitamin E+STZ	100	61.7±3.44	54.9±4.74	44.5±2.62[c]
Gliclazide+STZ	20	68.1±2.41	53.6±4.42	49.3±4.21[c]
Atorvastatin+STZ	10	54.2±3.34	45.6±2.56	29.3±3.54[b,c]

[a]p<0.001 as compared with non-diabetic control (Distilled water) group, [b]p<0.05 as compared with non-diabetic control (Distilled water) group, [c]p<0.001 as compared with diabetic control (STZ) group, One-way ANOVA followed by post-hoc Tukey's test.

Table 8: Effect of various drugs (x30 days) on 'Quadrant-time' of spatial navigation task in Streptozotocin (STZ) induced-diabetic rats (n=10).

Group	Dose and route (mg/kg, p.o.)	Time (Seconds) Mean±SEM		
		Day 1; IAL	Day15;QT-1	Day 30;QT-2
Distilled water (non-diabetic control)	Vehicle of *E. officinalis* and *M. koenigii*	43±4.67	61±4.44	69.4±4.51
Streptozotocin (diabetic control)	50, i.p.	55.5±2.76	34.2±3.43[a]	19.5±3.14[a]
***E. officinalis* Dose I+STZ**	250	59.6±2.65	41.7±3.97	48.2±4.73[a,d]
***E. officinalis* Dose II+STZ**	500	65.3±3.23	43.8±3.7[c]	55±3.26[a,d]
***M. koenigii* Dose I+STZ**	250	52.6±2.65	38.6±3.54[a]	43.3±2.74[a,d]
***M. koenigii* Dose II+STZ**	500	54.4±3.12	41.3±2.17[c]	48.3±2.28[d]
Melatonin+STZ	50	58.3±3.26	39.7±2.71[b]	66.2±3.21[d]
Vitamin E+STZ	100	49.5±2.23	42.6±2.33[c]	34±3.04[a]
Gliclazide+STZ	20	51.6±3.58	41.1±3.09[b]	42.1±2.56[a, b]
Atorvastatin+STZ	10	56.3±2.32	40.6±4.03	44.5±2.19[a,b]

[a]p<0.001 as compared with non-diabetic control (distilled water) group, [b]p<0.01 as compared with non-diabetic control (distilled water) group, [c]p<0.05 as compared with non-diabetic control (distilled water) group, [d]p<0.001 as compared with diabetic control (STZ) group. One-way ANOVA followed by post-hoc Tukey's test.

Table 9: Effect of various drugs (x30 days) on brain levels of MDA and GSH in Streptozotocin (STZ) induced Diabetic rats (n=10).

Group	Dose and route (mg/kg, p.o.)	MDA (nmol/g wet brain tissue) Mean±SEM	GSH (µg/g wet brain tissue) Mean±SEM
Distilled water (Non-diabetic Control)	Vehicle of *E. officinalis* and *M. koenigii*	191.4±12.45	364.3±11.76
Streptozotocin (Diabetic Control)	50, i.p.	346.8±16.76[a]	192.4±14.38[a]
***E. officinalis* Dose I+STZ**	250	252.4±15.88[c]	296.3±16.71[c]
***E. officinalis* Dose II+STZ**	500	234.2±12.32[c]	322.7±11.14[c]
***M. koenigii* Dose I+STZ**	250	265.8±10.76[b,d]	301.4±13.61
***M. koenigii* Dose II+STZ**	500	241.3±13.33 [c]	312±12.79[c]
Melatonin+STZ	50	220.6±13.34 [c]	259.3±6.03[a]
Vitamin E+STZ	100	191.7±12.45[c]	334.3±11.54[c]
Gliclazide+STZ	20	246.3±11.92[c]	241.2±7.65[a]
Atorvastatin+STZ	10	284.7±14.43 [a]	252.6±12.39[a,e]

[a]p<0.001 as compared with non-diabetic control (Distilled water) group, [b]p<0.01 as compared with non-diabetic control (Distilled water) group, [c]p<0.001 as compared with diabetic control (STZ) group, [d]p<0.01 as compared with diabetic control (STZ) group, [e]p<0.05 as compared with diabetic control (STZ) group, One-way ANOVA followed by post-hoc Tukey's test

Table 10: Effect of various drugs (x30 days) on brain levels of AChE in Streptozotocin (STZ) induced-diabetic rats (n=10).

Group	Treatment and route	AChE activity (mol/min/g protein) Mean±SEM
Distilled water (non-diabetic control)	Vehicle of *E. officinalis* and *M. koenigii*	162.2±13.65
Streptozotocin (diabetic control)	50, i.p.	267.3±12.29[a]
***E. officinalis* Dose I+STZ**	250	209.5±5.76[a,d]
***E. officinalis* Dose II+STZ**	500	196.1±12.43[a,c]
***M. koenigii* Dose I+STZ**	250	216.7±14.56[a]
***M. koenigii* Dose II+STZ**	500	197.4±12.71[a,c]
Melatonin+STZ	50	184.3±10.32[a,b]
Vitamin E+STZ	100	226±13.22[a]
Gliclazide+STZ	20	238.7±11.54[a]
Atorvastatin+STZ	10	242.1±13.67[a]

[a]p<0.001 as compared with non-diabetic control (distilled water) group, [b]p<0.001 as compared with diabetic control (STZ) group, [c]p<0.01 as compared with diabetic control (STZ) group, [d]p<0.05 as compared with diabetic control (STZ) group, One-way ANOVA followed by post-hoc Tukey's test.

Retention of step-down latency (SDL) in continuous avoidance apparatus

At day '15', "significant decrease**"** in retention of SDL was seen in diabetic control group II (p<0.05) as compared to group I. Group IV (p<0.001) showed "significant increase**"** in retention of SDL as compared to Group II.

At day '30', "significant decrease**"** in retention of SDL was seen in Group II (p<0.05) when compared to Group I. In comparison with Group II; the groups which showed "significant increase**"** in retention of SDL are Group III (p<0.05), Group IV (p<0.001), Group VI (p<0.05), Melatonin group (p<0.05), Vitamin E group (p<0.01), Group VII (p<0.01), Group VIII (p<0.05) (Table 6).

Retention of spatial navigation task in Morris Water Maze

No significant difference was seen in IAL in any group at day '1 and '15' compared to Group I.

At day '30', "significant increase**"** in RL-2 was seen in Group II ($p<0.001$) as compared to Group I. In comparison with Group II; the groups which showed "significant decrease**"** in RL-2 were Group III and IV ($p<0.001$), Group V and VI ($p<0.05$), Melatonin group ($p<0.001$), Vitamin E group ($p<0.001$), Group VII ($p<0.001$), Group III ($p<0.001$) (Table 7).

'Quadrant-time' (QT) in spatial navigation task in Morris Water Maze

No significant difference was seen in any group in IAL at day '1 compared to No treatment group 'I'.

At day '15', "significant decrease**"** in QT-1 was seen in Group II ($p<0.001$) as compared to Group I.

At day '30', "significant decrease**"** in QT-2 was seen in Group II ($p<0.001$) as compared to Group I. In comparison "significant increase**"** in QT-2 were seen in Group III and IV ($p<0.001$), Group V and VI ($p<0.001$) and Melatonin group ($p<0.001$) (Table 8).

Effect of various drugs (x30 day) on biochemical parameters

MDA and GSH levels in brain

In streptozotocin (STZ) induced diabetic group II, "significant increase**"** in brain MDA levels were seen ($p<0.001$) as compared to Group I. In comparison to Group II; the groups which showed "significant decrease**"** in brain MDA levels were - Group III and IV ($p<0.001$), Group V ($p<0.05$), Group VI ($p<0.001$), Melatonin group ($p<0.001$), Vitamin E group ($p<0.001$) and Group VII ($p<0.001$).

"Significant decrease**"** in brain GSH levels among diabetic rats was seen in Group II ($p<0.001$) as compared to Group I. In comparison to Group II; the groups which showed "significant increase**"** in brain GSH levels were Group III and IV ($p<0.001$), Group VI ($p<0.001$), Vitamin E group ($p<0.001$) and Group VIII ($p<0.05$) (Table 9).

AChE levels in Brain

Among streptozotocin (STZ) induced diabetic group II, brain AChE levels were found to be "significantly increased**"** ($p<0.001$) as compared to Group I. Group III ($p<0.05$), Group IV ($p<0.01$) and Group VI ($p<0.01$) showed "significant decrease**"** in brain AChE levels as compared to group II.

However, Group V, Melatonin group, Vitamin E group, Group VII and VIII did not show any significant change in brain AChE levels when compared to Group II (Table 10).

DISCUSSION

DM is an important risk factor for the development of cognitive impairment through several mechanisms. Further it has been documented that uncontrolled hyperglycaemia results in increased oxidative stress.[14]

The generation of oxygen free radicals (OFR) are supposed to be involved in the damage to endothelium, which is responsible for the progression of atherosclerosis. In addition to endothelial dysfunction, the central role of impaired lipid metabolism (lipid oxidation) is well established in the development of atherosclerosis. Advanced glycation end products (AGEs), formed as a result of prolonged hyperglycaemia, play a significant role in this process. One of the ways in which AGEs contribute to the development of atherosclerosis is by converting low-density lipoprotein (LDL) cholesterol to oxidized LDL (ox-LDL) cholesterol, which promotes its uptake by macrophages. This leads to the development of so-called "foam cells" and the subsequent formation of atherosclerotic plaques. Higher levels of oxidized cholesterol have been detected in patients with poorly controlled DM compared with those who achieve good glycaemic control.[15]

Increased formation of reactive oxygen species is an important feature of the diabetic endothelial cell dysfunction. This results in part from uncoupling of endothelial nitric oxide synthase such that it generates superoxide anion in addition to NO, forming peroxynitrite, a damaging molecule. This not only helps to explain the impairment of endothelial vasodilator mechanisms, but also increased progression of vascular disease. Many of these cellular abnormalities can be prevented by adequate scavenging of oxygen derived free radicals.

Cognitive dysfunction and dementia have recently been proven to be common (and under recognized) complications of diabetes mellitus (DM). In fact, several studies have demonstrated that alterations in glucose homeostasis increases risk for developing cognitive decline and dementia, including not only vascular dementia, but also Alzheimer's disease (AD).[16]

DM being an atherogenic risk factor, it may increase the risk of dementia. Decreased cholinergic transport across the blood-brain barrier observed in diabetic animals may exacerbate cognitive impairment.[17]

The actual mechanism of diabetes causing brain damage has not been completely elucidated but it appears to be a multifactorial process involving chronic metabolic and vascular disturbances resulting from fluctuations in blood

glucose levels.[18] It has been demonstrated that hyperglycaemia induces oxidative stress in various brain regions which perhaps results in cognitive impairment.[19]

In the current study melatonin, Vitamin E, Gliclazide, Atorvastatin, two plants—*E. officinalis* (Amla) and *M. koenigii* (Curry leaves) have been used to evaluate the antioxidant potential and their role in modulation of cognitive function. The study involved evaluation of various parameters like blood glucose level, lipid profile, malondialdehyde, reduced glutathione and acetylcholinesterase level in diabetic rats.

Fasting blood glucose levels and lipid profile were measured as the biochemical indicator of diabetes and hyperlipidaemia respectively. 30 days treatment of the diabetic rats with *E. officinalis*, *M. koenigii* and Gliclazide significantly decreased the blood glucose levels. The findings indicate that hyperglycaemia caused by STZ is significantly decreased by *E. officinalis* and *M. koenigii* and this effect is comparable to that of Gliclazide.

Diabetic control rats after 30 days showed changes in serum lipid profile. There was rise in serum total cholesterol, triglycerides and LDL cholesterol levels and fall of HDL cholesterol levels. In the present study *E. officinalis* (500 mg/kg), *M. koenigii* (250 and 500 mg/kg) and Atorvastatin resulted in significant decrease in serum total cholesterol, triglycerides and LDL levels and increase in HDL level. Our results are in accordance with previous studies.[20,21]

Transfer latency on elevated plus maze, step-down latency on passive avoidance apparatus and spatial navigation task in Morris-water maze were used to assess the behavioural parameters of learning and memory. Brain levels of MDA and GSH were measured as an analysis of oxidative stress and AChE levels in brain were measured as the biochemical parameter of cognition and memory. The transfer latency (TL) was significantly increased in both acquisition and retention components of TL in diabetic rats, suggestive of learning and memory impairment in these animals. 30 days treatment with high dose *E. officinalis* (500 mg/kg) significantly decreased the acquisition of TL, whereas *E. officinalis* (500 mg/kg), *M. koenigii* (500 mg/kg) and Melatonin significantly decreased the retention component of TL. The findings indicate that the learning and memory impairment in diabetic rats is significantly reversed and/or decreased by *E. officinalis* (500 mg/kg), *M. koenigii* (500 mg/kg) and this effect was comparable to that of Melatonin.

Acquisition and retention components of SDL was decreased significantly in diabetic rats, suggestive of learning and memory impairment. *E. officinalis*, *M. koenigii*, Melatonin, Gliclazide and Atorvastatin significantly increased acquisition and retention component, whereas Vitamin E increased retention component only. The above findings indicate that the learning and memory impairment caused by diabetes was significantly reversed and/or decreased by *E. officinalis*, *M. koenigii* and this effect was comparable to that of Melatonin, Gliclazide and Atorvastatin.

The spatial navigation task in Morris water maze was used to assess learning and memory behaviour, the test assessed two components of spatial navigation, viz. retention latency at day 15 and day 30 (RL-1 and RL-2 respectively) and time spent in target quadrant (QT-1 and QT-2 respectively). Findings indicates that diabetes significantly increased both RL-1 and RL-2, and significantly decreased both QT-1 and QT-2. RL-2 and QT-2 was reversed with all the drugs used in the study. The results indicate that the learning and memory impairment was significantly reversed and/or decreased by *E. officinalis*, *M. koenigii* and this effect was comparable to that of Melatonin, Vitamin E, Gliclazide and Atorvastatin. The above findings of behavioural tests were similar to other studies.[18,22,23]

The assessment of biochemical parameters of oxidative stress were done by measuring brain MDA and GSH levels. Diabetic rats showed significant increase in brain MDA and decrease in GSH level. *E. officinalis*, *M. koenigii*, Melatonin, Vitamin E and Gliclazide caused significant decrease in brain MDA and significant increase in brain GSH levels.

Diabetic rats showed significant increase in AChE activity whereas *E. officinalis*, *M. koenigii* and Melatonin significantly decreased the AChE activity. Earlier studies have shown the direct correlation between the AChE activity and cognitive dysfunction.[24-26]

The results of biochemical tests were in agreement with other studies which have verified the cognitive impairment in diabetic rats.[26-28]

In the present study, it was observed that *E. officinalis* and *M. koenigii* reversed and/or decreased the enhanced AChE activity (caused by diabetes) thus improving the learning and memory behaviours as indicated by behavioural tests conducted. The oxidative stress parameters (MDA and GSH) were also positively modulated by *E. officinalis* and *M. koenigii* in form of decreased oxidative damage to neurons.

This neuroprotective and neuro-modulatory effect of *E. officinalis* and *M. koenigii* resulting in improvement of learning and memory in diabetes might be exploited in diabetic patients with cognitive dysfunction. However further studies particularly clinical studies are required to determine the efficacy and safety of extract of *E. officinalis* and *M. koenigii* in diabetic patients who are suffering from cognitive dysfunction.

CONCLUSION

The results of the present study conclusively showed that both *E. officinalis* and *M. koenigii* have antioxidant activity in experimental diabetes mellitus. Both these

drugs were effective in slowing the progression of cognitive impairment. The antioxidant activity of these drugs might be partially responsible for their effect in delaying cognitive impairment, besides this both the herbal extracts have got an anti-hyperglycaemic and hypolipidemic activity. However, future studies are required to provide an elaborate view on use of these two products in clinical medicine for treatment of diabetes and its neurological sequel.

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