EVALUATION OF *IN-VITRO* ALDOSE REDUCTASE AND ANTI-DIABETIC ACTIVITIES OF *MURRAYA KOENIGII* SEEDS

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Abstract

Aim: The purpose of the current study was to assess *in-vitro* aldose reductase and the anti-diabetic properties of *Murraya koenigii* (MK) seeds on Streptozotocin (STZ)-induced diabetic rats.

Methodology: The *in-vitro* antioxidant capacity of the prepared extract was compared with that of ascorbic acid. Then *in-vitro* aldose reductase activity of extract was assessed employing quercetin as a reference in rat lens and kidney tissue samples. This was followed by the *in-vivo* antidiabetic activity (200 mg/kg and 400 mg/kg body weight) of methanolic extract of *Murraya koenigii* (MEMK) seeds was carried out in STZ induced rats for a study period of 60 days. During this period, fasting blood glucose (FBG) levels were assessed. The serum parameters, anti-oxidant and urine parameters were all evaluated at the end of the study. The histopathological investigation was carried out on rat kidney, eye lens and pancreas.

Results: Substantial *in vitro* antioxidant activity was detected for *M. koenigii* (MK) extract. Methanolic extract of MK showed significant *in vitro* inhibitory activity against aldose reductases in rat lens, kidney and human homogenate samples. In the antidiabetic study, the changes seen in the FBG levels, serum parameters, antioxidant levels, and urine parameters nearly returned to normal. Microscopic examinations of the pancreatic cells of STZ-induced diabetic rats revealed deterioration and atrophy. Inflammation in the ciliary muscles of the lens was seen in STZ-induced diabetic rats along with tubular dilatation, degeneration, and haemorrhages in kidney; these histopathological defects were delayed following administration of MK extract. The extract effectiveness was on par with that of the well-known hypoglycaemic medication glibenclamide.

Conclusion: These results support *in-vitro* aldose reductase effect and antidiabetic effect of MK seeds and further studies to develop Nutraceutical from MK seed will be useful.

Keywords: aldose reductase activity, anti-diabetic activity, anti-oxidant activity, Diabetes, Murraya koenigii, Streptozotocin.

1. Introduction

The global epidemic of type 2 diabetes (T2DM) has sparked research into new compounds, therapeutic delivery methods, and targets in an effort to improve clinical outcomes. Given the population expansion, age, urbanization, an upsurge of obesity, and lack of physical activity, DM is becoming more prevalent globally. Young to middle-aged individuals, elderly people, and all other age groups are experiencing excessive increases in diabetes. The economic and health of a country might suffer long-term consequences from this, particularly in emerging nations. By 2040, the International Diabetes Federation (IDF) predicts that there will be 642 million diabetic individuals worldwide (Yan Zheng *et.al*). Improving glycaemia to a level that is close to normal is the main objective of managing DM. Over 50% of patients develop vascular problems and poor glycemic control. Diabetes increases the risk of many complications like diabetic retinopathy, neuropathy, nephropathy etc. are the most common cause of blindness, kidney failure, heart attacks and stroke. An enzyme called aldose reductase plays a major role in the development of complications (Ohta, M.*et.al,* Crabbe, and M.*et.al,* Varma, S. D. *et.al*). According to WHO, from 2000 to 2019 there was an increase in diabetic mortality rate because of diabetes complications.

Hence, it is crucial to create innovative anti-diabetic agents with aldose reductase inhibitor activity that do not compromise patient safety or cause problems to expand and worsen. The management of diabetes remains a significant issue even with the remarkable progress in biomedical development and growing understanding and possibly successful therapeutic options. In order to address this issue, researchers from a variety of fields are working diligently to find a safe and secure, yet practical way to treat DM by evaluating naturally derived derivatives acting on novel target proteins in addition to a proper analysis of the mechanisms involved of the active compounds, both of which are useful for further validating a number of new molecular drug candidates.

The Rutaceae family plant *Murraya koenigii* (curry plant), originally from India, is now found throughout most of Southern Asia. The plant's leaves are commonly referred to as "curry leaves" and have been utilized as one of the key components in cooking. Additionally, *M. koenigii* has been utilized as a traditional remedy. Although the plant's hypoglycemia properties have been documented in the literature, its seeds' anti-diabetic properties have not yet been studied (Arulselvan et al., 2006; El-amin et al., 2013; Dineshkumar et al., 2010). Plant produces small white coloured flowers which can self-pollinate and produce berry fruits containing one or two seeds. Fruiting starts in July to august. Seeds usually used for germination for next crop but utilize small quantity for this purpose (Jain, V. et al., 2012). Remaining the yield MK seeds can be effectively utilized if any nutraceutical is prepared from them. Therefore, this study evaluated the anti-diabetic properties as well as aldose reductase activity of *Murraya koenigii* (MK) seeds in streptozocin (STZ)-induced diabetic rats so that the study support to develop nutraceutical from MK seeds, that will be helpful to treat the diabetes and avoid/delay the diabetic complications in patients.

2. Materials and Methods

2.1 Plant extraction

Extraction of *Murraya koenigii* seeds

Murraya koenigii seeds were collected and authenticated by Dr. K.Madhav Chetty, Assistant Professor, Sri Venkateswara University and the voucher specimen (voucher number 1191)

was deposited in Herbarium of Department of Botany, Sri Venkateswara University, Tirupati. *Murraya koenigii* seeds were well cleaned with clean tap water, let to air dry at room temperature for 10 to 15 days, then were powdered and extracted in methanol using cold maceration process in three consecutive cycles. The experiment was performed using this extract. The extract was filtered and then dehydrated with rotavapor, yielding a dark brown viscous liquid that was stored in a desiccator to be used later.

2.2 Phytochemical Screening

The prepared extract were tested for the presence of different phytochemicals (Yadav et al., 2011). The reagents were newly prepared, and all of the chemicals and solvents utilized for the study were purchased from SD-fine Chemicals, India.

2.3 *In vitro* anti-oxidant activity

2.3.1 DPPH scavenging activity

As per the technique proposed by Brand-Williams et al., the antioxidant activity of the extract was assessed based on the scavenging activity of the stable 1, 1- diphenyl 2-picrylhydrazyl (DPPH) free radical with minor modifications (Brand-Williams et al., 1995). The MEMK scavenging activity was estimated by using different concentrations (20, 40, 80, 160, and 320 µg/mL). L-Ascorbic acid (1-100 µg/mL) was used as reference standard. Absorbance was measured at 517 nm. The inhibition percentage was calculated by using the following formula.

$$Inhibition\ \% = \frac{\mathrm{Ac} - \mathrm{As}}{Ac} \times 100$$

Where Ac is the absorbance of the control As is the absorbance of the sample

2.3.2 Superoxide Radical Scavenging Activity

The scavenging ability of the methanolic extract towards chemically produced superoxide radicals was assessed by spectrophotometric analysis of the product on reduction of nitro blue tetrazolium (NBT) (Chou et al., 2009). By using 1 mL of the test solution in different concentrations (20, 40, 80, 160, and 320 µg/mL) the absorbance was measured at 560 nm. The following formula was used to determine the percentage of scavenging activities:

$$\%\ \text{Superoxide radical Scavenging activities} = 1 - \frac{\mathrm{As}}{Ac} \times 100$$

Where Ac is the absorbance of the control As is the absorbance of the sample

2.3.3 Hydroxyl Radical Scavenging Activity

This was tested using the procedure that Elizabeth and Rao outlined (Elizabeth et al., 1990). The technique relies on quantifying the 2-deoxyribose breakdown product by condensation with thiobarbituric acid (TBA). Scavenging activity was estimated with different concentrations of the test sample or reference (20, 40, 80, 160, 320 µg/mL). The absorbance was measured at 562 nm. The percentage inhibition was calculated by using the following formula.

$$Inhibition\,\% = \frac{A_0 - A_1}{A_0} \times 100$$

where

A_0 was absorbance of the control (without extract),

A_1 was the absorbance in the presence of the extract

2.3.4 Hydrogen peroxide Radical Scavenging Activity

The methanol extracts' ability to scavenge hydrogen peroxide (H_2O_2) was assessed using the previously described procedure (Ebrahimzadeh et al., 2010; Haro-Vicente et al., 2006). It was performed with different concentrations (20,40,80,160,320 µg/mL) of test solution. Ascorbic acid was used as the control in the comparison as µg/g dry weight. The following formula was used to determine the % of hydrogen peroxide scavenging capacity of the extract.

$$Inhibition\,\% = \frac{\mathrm{Ac} - \mathrm{As}}{Ac} \times 100$$

2.4 *In-vitro* aldose reductase inhibitor assay

2.4.1 Sample Preparation

2.4.1.1 Preparation of crude rat lens homogenate

Wistar albino male rats of around 150 gk weight were used for the investigation. The eyes of slain animals were removed, and after being enucleated by the posterior method, the lenses were preserved until required. By following the Hayman and Kinoshita (1965) standard procedure (Hayman et al., 1965), the rat lens supernatant was collected. It was then homogenized with 0.1 M sodium phosphate buffer, pH 6.2, using a tissue homogenizer (Zentrifugen, Micro 220R, Hettich, Germany). Crude rat lens aldose reductase (AR) enzyme was made from the supernatant by centrifuging the homogenate at 16,000 rpm and 4 °C (Jaslin et al., 2010).

2.4.1.2 Preparation of rat kidney homogenate

Rats were excised ventrally, and both kidneys were removed and sliced into tiny pieces (Cerelli et al., 1986). Kidneys were then homogenized in the same manner as lens homogenate in order to get rat kidney AR enzyme.

2.4.2 Protein determination in sample

By employing crystalline albumin as a reference, the Lowry technique (Lowry et al., 1951) was used to determine the protein content of the sample. Plotting was done using the 0-0.2 mg/mL standard calibration curve. Then, using a standard calibration curve, the protein content was discovered to be 1.44 ± 0.02 (Mean ± SD, n=3) mg/mL for the lens and 8 ± 0.01 (Mean ± SD, n=3) mg/mL for the kidney.

2.4.3 Determination of aldose reductase enzyme activity

According to Hayman and Kinoshita's approach, AR enzyme activity was measured (Hayman et al., 1965). The source of the enzymes was derived from crude extracts of albino rat lenses. Each reaction cuvette received the whole set of ingredients, and AR enzyme activity was measured after five minutes. Before adding glyceraldehyde and the substrate to the cuvette, the absorbance was read at 340 nm. After maintaining the incubation duration of three minutes, the reduction in absorbance was observed at 340 nm. The background modifications were done while a blank solution was created without the substrate under the identical circumstances.

The average aldose reductase activity in rat lens homogenate calculated as 1.135±0.058 (Mean± SD, n=5) nmole NADPH oxidized /min/ mg protein. The average aldose reductase activity in rat kidney homogenate calculated as 0.125 ± 0.48 (Mean ± SD, n=10) nmole NADPH oxidized /min/ mg protein.

2.4.4 *In vitro* aldose reductase inhibitor assay

This method is based on the reduction of DL- glyceraldehyde as a substrate to glycerol by enzyme which is aldose reductase during the oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) to NADP+. The oxidation of NADPH was spectrophotometrically followed at 340 nm using spectrophotometer. In our study, albino rat lens crude extracts were taken as the enzyme source. All of the components were put into the reaction mixture with the given order was shown in table. Determination of aldose reductase enzyme activity was applied for five minutes to each reaction cuvette. Absorbance was measured immediately at 340 nm before substrate, glyceraldehyde was added in cuvette, three minutes incubation time was maintained and the decrease in absorbance was measured once again at 340 nm. Repeat the same process with addition of plant extract to determine the aldose reductase inhibitor activity.

The assay mixture contained final concentrations of $6x10^{-5}$ M DL-Glyceraldehyde as substrate solution, $1x10^{-4}$ M NADPH prepared freshly, 1.43 mg/ml protein containing crude enzyme solution, 270 mM Li_2S0_4, and 30 mM potassium phosphate buffer pH 6.7 in a final volume of 1.0 mL, is shown in Table. The specific activity was written as nmole NADPH oxidized per minute per mg protein per mL. One unit of enzyme was defined as amount of enzyme catalyzing the oxidation of 1 nmole NADPH per minute (Hayman et al., 1965).

$$\text{Specific activity (nmol.}\,\text{min}^{-1}.\,(mg/ml)^{-1} = \frac{Sample\ OD\ - Blank\ OD}{\varepsilon^{340}\ (NADPH).\,[Protein]}$$

Extinction coefficient of NADPH (6220 mM^{-1})

Table 1: The components of assay mixture for aldose reductase

Components	Volume Added (µL)	Final Concentration
Glyceraldehyde	40	$6x10^{-5}$ M
NADPH	50	1×10^{-4} M
Enzyme Solution	100	1.43 mg/mL
Li2S04	135	270mM
pH 6.7 Phosphate Buffer	600	30 mM

dH_20	To complete final volume to 1 mL	-
Total volume	1 mL	-

2.5. Experimental Animals

For this investigation, healthy Wistar rats weighing 160–200g were obtained from NIN. Prior to beginning the experiment, animals were kept in groups for a week to become acquainted to the laboratory environment. Animals were given standard food and adequate access to water. The animals were starved for 12 hours before experiment, allowing access to water. Following CPCSEA requirements, all experimental procedures were completed. The experimental protocol has been approved by the Institutional Animal Ethics Committee (I/IAEC/AGI/038/2017WR♀ + ♂).

2.6. Induction of Diabetes

The STZ (60 mg/kg) was dissolved in 0.1M cold sodium citrate buffer, adjusted the PH to 4.5, and given intraperitoneally to overnight-fasted rats. Animals were given a 20 percent w/v glucose solution after receiving STZ injections to avoid hypoglycaemia. Glucometer (ONETOUCH select plus simple glucometer strips) was used to measure the fasting blood glucose levels (FBG) after 72 hours. Rats with blood sugar levels below 250 mg/dl were barred from the experiment, while those with levels above 250 mg/dl were chosen for the diabetes investigation and divided into groups.

2.7. Experimental Study Design

Rats were divided into five groups containing six rats in each group and were given the following treatment for a period of 60 days.

Group –I : Normal control rats, given saline only.

Group-II : Diabetic control rats, given saline daily after diabetic induction.

Group-III : Diabetic rats given MEMK (200 mg/kg b.w) once daily.

Group-IV : Diabetic rats given MEMK (400 mg/kg b.w) once daily.

Group-V : Diabetic rats given Glibenclamide (10 mg/kg b.w) once daily.

On 60th day, after the dose of treatment with extract, animals were sacrificed and blood samples were collected for the analysis of various parameters and organs like eyes, kidneys and pancreas were collected and stored for histopathological and western blot studies.

2.8. Histopathology studies

Histopathology was carried out for pancreas, kidney and eye balls to check the degeneration and prevention of damage by the extract.

3. Results

3.1 Phytochemical Screening

In order to determine the presence of chemical constituents, phytochemical tests were performed, which revealed the presence of phytoconstituents in methanol extract (Table 1)

Table 2: Phytochemical screening of methanol extract of *Murraya koenigii* (MEMK) seed.

S. No.	Phytochemical test	MEMK
1	Alkaloids	+
2	Glycosides	–
3	Saponin	–
4	Phenols	+
5	Steroids	+
6	Flavonoids	+
7	Terpenoids	+
8	Tannin	–
9	Carbohydrates	–

+ = Present, - = Absent

3.2 *In vitro* anti-oxidant activity

3.2.1 DPPH Scavenging Activity

The extract showed a concentration-dependent antiradical activity by inhibiting DPPH radical with IC_{50} value of 117.42±3.2 (Table 2).

Table 3: DPPH scavenging activity of *Murraya koenigii* Methanolic extract

Conc. of extracts (µg/mL)	MEMK (Mean ± SD)	Ascorbic acid (Mean ± SD)
20	19.22±1.2	48.00±1.2
40	35.14±1.4	88.08±1.4
80	45.32±1.3	90.68±2.4
160	55.14±2.2	93.63±2.6
320	68.14±1.4	94.21±1.2
IC_{50}	117.42±3.2	18.5±1.5

3.2.2 Superoxide Radical Scavenging Activity

The radical scavenging activity of MK extract was proportional to the concentration with IC_{50} value 147.25 µg/mL (Table 3). These results indicated that the tested extract had a notable

effect on scavenging of superoxide when compared with ascorbic acid, which was used as positive control

Table 4: Superoxide radical scavenging activity of *Murraya koenigii* Methanolic extracts (values represent means ±.S.D, n=3)

Conc. of extracts (µg/mL)	MEMK	*Ascorbic acid*
20	41.34±1.2	56.87±0.24
40	51.12±0.6	74.46±0.74
80	66.24±1.2	80.72±0.4
160	74.45±2.1	84.41±1.57
320	82.22±1.2	86.25±2.3
IC_{50}	147.25	53.5

3.2.3 Hydroxyl Radical Scavenging Activity

The extract showed a moderate hydroxyl radical-scavenging activity, as tabulated in Table 4. The % inhibition was increased with increasing concentration of the extract. Ascorbic acid, a natural antioxidant was used as a positive control for comparison

Table 5: Hydroxyl radical scavenging activity of *Murraya koenigii* Methanolic extract (values represent means ±.S.D, n=3)

Conc.of extracts (µg/mL)	MEMK	*Ascorbic acid*
20	35.11±1.0	55.61±1.0
40	49.12±1.3	65.31±0.6
80	59.13±1.4	76.25±0.4
160	67.34±1.4	82.11±1.0
320	72.14±1.2	91.22±1.3
IC_{50}	180.22	67.8

3.2.4 Hydrogen peroxide Radical Scavenging Activity

H2O2 scavenging activity of the plant extract was significant compared to that of the standard ascorbic acid. Table 5 shows the hydrogen peroxide scavenging activity of MK extract in comparison with Ascorbic acid standard.

Table 6: Hydrogen peroxide radical scavenging activity of *Murraya koenigii* Methanolic extract (values represent means ±.SD, n=3)

Conc.of extracts (µg/mL)	MEMK	*Ascorbic acid*
20	21.43±0.97	50.00±0.10
40	30.00±1.70	61.90±0.72
80	39.50±0.78	72.63±0.75
160	64.80±1.08	84.53±1.00
320	82.70±1.01	91.66±0.76
IC_{50}	62.94	18.20

3.3 *In-vitro* Aldose reductase inhibitor activity

Methanolic extract of MK showed significant inhibitory activity against aldose reductases in rat lens, kidney and human homogenate samples, as shown in Table 7. Quercetin was used as a reference.

Table 7: Aldose reductase activity of *Murraya koenigii* in Methanolic extracts

Plant extract	IC_{50} (RLAR)	IC_{50}(RKAR)	IC_{50}(HRAR)
MEMK	84.31 ± 0.99	73.22 ± 0.99	45.86 ± 0.52
Quercetin	4.65 ± 0.05	5.61±0.02	2.79 ± 0.21

(RLAR- Rat lens aldose reductase; RKAR- Rat kidney aldose reductase; HRAR- Human recombinant aldose reductase; IC_{50} -50% inhibitory concentration).

3.4 *In vivo* antidiabetic efficacy

3.4.1 Effect of plant extracts on FBG

The extract showed significant (P<0.001) reduction in FBG levels in dose-dependent manner. A concentration of 400 mg/kg was found to show optimum activity; on 60th day, FBG levels were restored to normal (Table 8).

Table 8: Fasting Blood Glucose levels of *Murraya koenigii* in Methanolic extract

	Fasting Blood Glucose level mg/dl					
Groups	**1st day**	**14th day**	**28th day**	**42nd day**	**56th day**	**60th day**
Control	87± 5.099	87.75± 13.43	86.75± 9.251	80.75± 4.924	84.75± 2.754	81.50± 2.464

Diabetic control	305.8± 18.46	386.7± 10.41$^{###}$	383± 2.646$^{###}$	378.7± 5.033$^{###}$	353.3± 68.7$^{###}$	358.3± 20.11$^{###}$
MEMK (200 mg/kg, p.o)	284.8± 12.92 ^{ns}	272± 8.981***	243.5± 7.047***	165.3± 7.974***	112.8± 6.801***	109.8± 6.238***
MEMK (400 mg/kg, p.o)	272.8± 6.185**	237± 10.25***	192± 4.697***	129± 1.826***	94.75± 2.986***	87.25± 2.630***
Glibenclamide (10 mg/kg, p.o)	219.0± 7.000***	120.8± 4.349***	106± 6.164***	89.25± 3.304***	79.75± 5.188***	79.50± 2.646***

Data were expressed as mean ± SD (n=6) and analysed by one way ANOVA followed by Tukey's multiple comparison test. $^{###}$P<0.001 as compared to control group. ***P<0.001, **P< 0.01, *P< 0.05 as compared to the diabetic control group.

3.4.2 Effect of plant extracts on serum parameters

Creatinine levels were increased in the diabetic control group when compared to the control group ($P < 0.001$), whereas the levels were decreased in MK low dose, high dose, Insulin levels were decreased in the diabetic control group ($P < 0.001$), when compared to the control group, these levels were increased in treatment groups ($P < 0.001$). HbA1C levels were increased in diabetic control ($P < 0.001$), when compared to the control group, the levels were decreased in treatment groups ($P < 0.01$). Blood urea nitrogen (BUN) levels were increased in the diabetic control group ($P < 0.001$), when compared to the control group, levels were increased in treatment groups ($P < 0.001$) (Table 9).

Table 9: Effect of *Murraya koenigii* in Methanolic extracts on serum parameters of diabetic rats

Groups	Creatinine (mg/dL)	Insulin	HbA1C	BUN (mg/dL)
Control	0.566±1.528	1.83±0.10	5.233±0.568	18.63±0.51
Diabetic control STZ 60mg/kg, i.p	1.567±0.32$^{###}$	0.33±0.03$^{###}$	10.90±0.56$^{###}$	54.53±1.124$^{###}$
MEMK (200 mg/kg, p.o)	1.1±0.10*	1.10±0.11***	7.967±0.208***	31.15±2.973***
MEMK (400	0.8±0.10***	1.79±0.047***	6.243±0.35***	22.33±0.83***

mg/kg, p.o)				
Glibenclamide (10mg/kg, p.o)	0.5±0.10***	1.89±0.026***	6.3±0.36***	20.50±1.291***

Data were expressed as mean ± SD (n=6) and analysed by one way ANOVA followed by Tukey's multiple comparison test. $^{\#\#\#}$P<0.001 as compared to control group. ***P<0.001, **P< 0.01, *P< 0.05 as compared to the diabetic control group.

3.4.3 Effect of plant extracts on lipid profile

Triglyceride levels was increased in diabetic control group (P < 0.001), when compared to control group, the levels were decreased in treatment groups (P < 0.001). HDL levels were decreased in diabetic control (P < 0.001), when compared with control group, these levels were increased in treatment groups (P < 0.001). Total cholesterol levels were increased in diabetic control (P < 0.001), when compared to control group, these levels were decreased in treatment groups (P < 0.001) when compared to diabetic control group. LDL levels were increased in diabetic control group (P < 0.001), when compared to control group, the levels were significantly decreased in all treatment groups (P < 0.001). VLDL levels were increased in diabetic control group (P < 0.001), when compared to control group, the levels were significantly decreased, restored to normal levels in treatment groups (P < 0.001) (Table 10).

Table 10: Effect of *Murraya koenigii* in Methanolic extracts on lipid profile of diabetic rats

Groups	**Triglyce rides**	**HDL**	**Total cholesterol**	**LDL**	**VLDL**
Control	94± 4.359	43.33 ±1.528	69.20± 7.663	95.67± 1.75	15.75± 0.64
Diabetic control STZ 60mg/kg, i.p	287.7± 39.55$^{\#\#\#}$	8.4 ±0.36$^{\#\#\#}$	155.3± 12.50$^{\#\#\#}$	177.8± 2.31$^{\#\#\#}$	36.13± 0.85$^{\#\#\#}$
MEMK (200 mg/kg, p.o)	181± 13.75***	17.23 ±2.937***	108.7± 4.509***	126.0± 5.06***	22.38± 0.47***
MEMK (400 mg/kg, p.o)	114.7± 8.505***	32.90± 1.277***	79.33± 6.658***	110.8± 2.13***	18.63± 0.47***
Glibenclamide (10mg/kg, p.o)	104.3± 7.767***	39.67± 0.763***	75± 2.646***	114.3± 2.16***	16.75± 0.50***

Data were expressed as mean ± SD (n=6) and analysed by one way ANOVA followed by Tukey's multiple comparison test. $^{\#\#\#}$P<0.001 as compared to control group. ***P<0.001, **P< 0.01, *P< 0.05 as compared to the diabetic control group.

3.4.4 Effect of plant extracts on anti-oxidant parameters

SOD levels were decreased in the diabetic control group ($P < 0.001$), when compared to the control group, levels were increased, restored to normal levels in all treatment groups ($P < 0.001$). Catalase levels were decreased in the diabetic control group ($P < 0.001$), when compared to the control group, whereas the levels were increased and restored to normal in all treatment groups ($P < 0.001$). GSH levels were decreased in diabetic control ($P < 0.001$), when compared to the control group, the levels were increased in treatment groups ($P < 0.001$), when compared to diabetic control group (Table 11).

Table 11: Effect of *Murraya koenigii* in Methanolic extracts on anti-oxidant parameters of diabetic rats

Groups	SOD	Catalase	GSH
Control	54.38±3.969	32.77±2.046	51.33±2.695
Diabetic control STZ 60mg/kg, i.p	36.29±3.170$^{\#\#\#}$	16.34±1.566$^{\#\#\#}$	31.021±3.144$^{\#\#\#}$
MEMK (200 mg/kg, p.o)	42.39±2.186 ns	25.55±1.921***	40.73±1.661**
MEMK (400 mg/kg, p.o)	55.05±5.010***	34.02±2.375***	49.52±0.833***
Glibenclamide (10mg/kg, p.o)	54.87±2.309**	30.82±0.83***	50.03±2.578***

Data were expressed as mean ± SD (n=6) and analysed by one way ANOVA followed by Tukey's multiple comparison test. $^{\#\#\#}$P<0.001 as compared to control group. ***P<0.001, **P< 0.01, as compared to the diabetic control group.

3.4.5 Effect of plant extracts on urinary parameters

Urinary pH was declined in diabetic control ($P < 0.001$), when compared to the control group, the pH was restored to normal in treatment groups ($P < 0.001$), when compared to diabetic control group (Table 12). Volume of urine output was increased in diabetic control group ($P < 0.001$), when compared to the control group. It was decreased after treatment with methanolic extracts and standard glibenclamide.

Table 12: Effect of *Murraya koenigii* in Methanolic extract on urinary parameters of diabetic rats

Groups	Urine PH	Urine volume
Control	6.275±0.17	9.150±0.79
Diabetic control STZ 60mg/kg, i.p	3.650±0.42$^{\#\#\#}$	46±2.944$^{\#\#\#}$
MEMK (200 mg/kg, p.o)	4.875±0.27***	16.25±1.708***

MEMK (400 mg/kg, p.o)	5.575±0.22***	14±1.826***
Glibenclamide (10mg/kg, p.o)	6.125±0.125***	10.20±1.304***

Data were expressed as mean ± SD (n=6) and analysed by one way ANOVA followed by Tukey's multiple comparison test. ***P<0.001 as compared to the diabetic control group.

3.5 Histopathological Studies

3.5.1 Pancreas (10X magnification)

Treatment with STZ caused degeneration of β cells along with atrophy of pancreatic cells in diabetic rats (negative control) (Figure 1). However, MK- and glibenclamide-treated groups exhibited noticeable improvement of the cellular injury, as apparent by the partial restoration of islet cells, hyperplasia and hypertrophy β-cells and growth in number of islet cells.

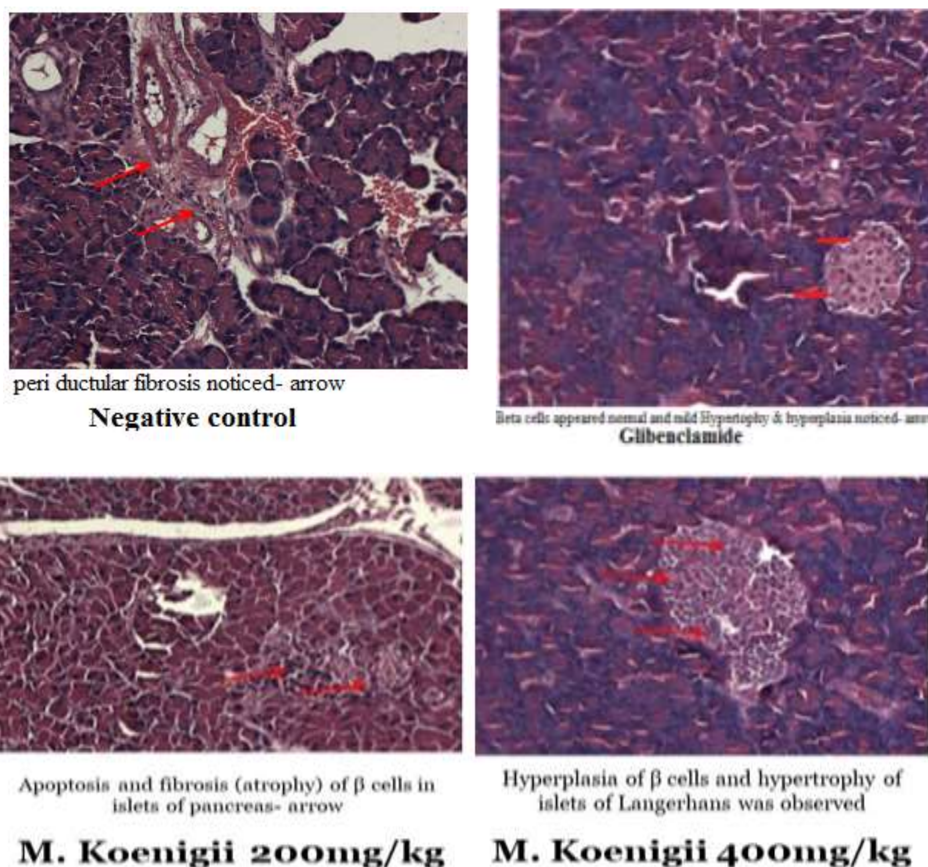

Figure 1: Histopathology of pancreas

3.5.2 Kidney (10X magnification)

Microscopic study of kidney showed dilation and degeneration of tubules in negative control group (Figure 2). Following treatment with MK extract, there was regeneration of tubules. At a concentration of 400mg/kg, the extract showed significant activity.

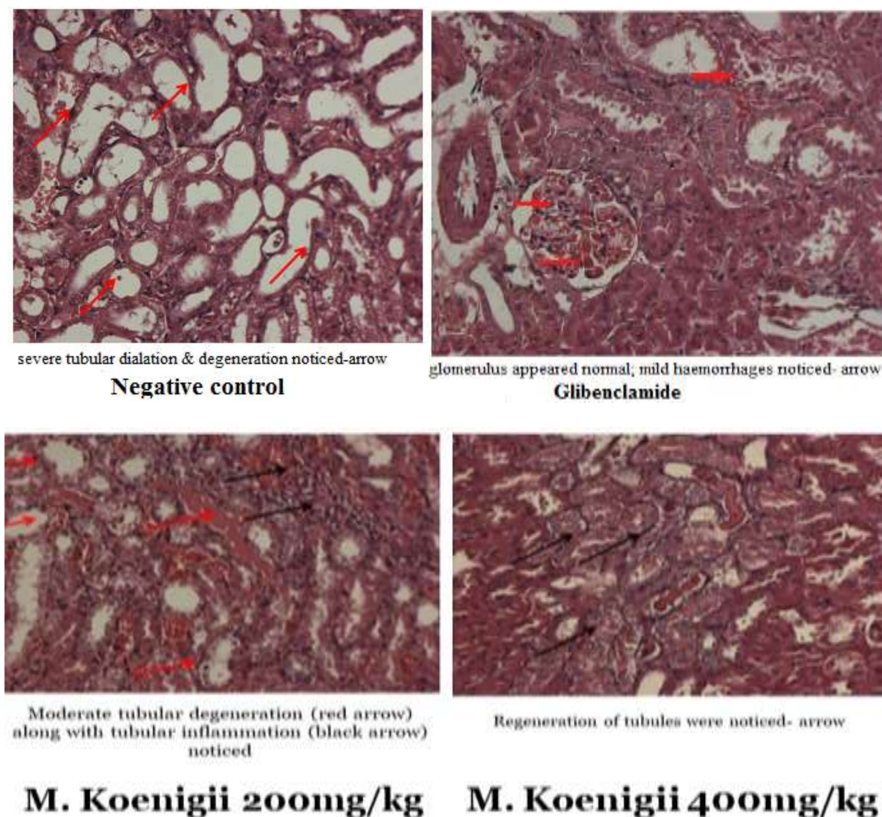

Figure 2: Histopathology of Kidney

3.5.3 Eye (10X magnification)

Histological analysis of eye from STZ-treated group showed degenerative changes of vacuoles in lens (figure 3). The damage to the lens were reversed in MK treated groups.

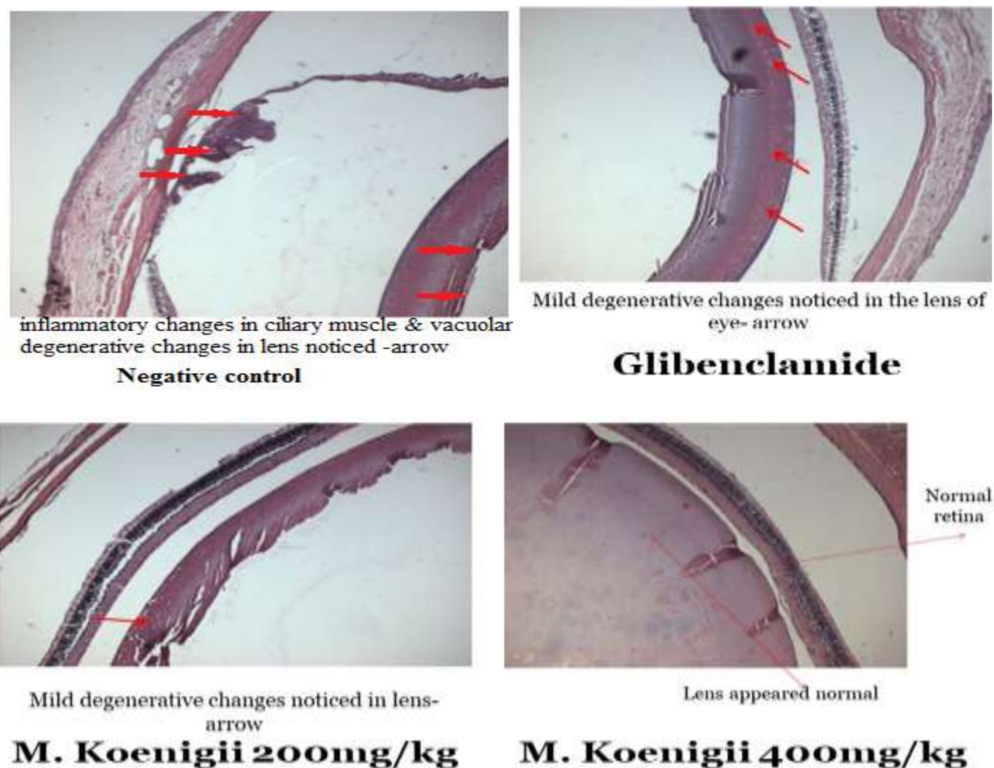

Figure 3: Histopathology of Lens

4. Discussion

The current study sought to determine if methanolic extract of *M. koenigii* seeds had any anti-diabetic effects in streptozotocin-induced diabetic rats.

A number of literature papers have linked the development of diabetes comorbidities like cataract, nephropathy, neuropathy, and retinopathy to AR production. Plant extracts have therefore been tested for AR inhibiting action in the current investigation. The high concentration of phenolic components in the plant may be responsible for the extracts' AR inhibiting action in rat lens and kidney tissue (Termentzi et al., 2008). It has been demonstrated that phenolic substances have potent inhibitory effects on rat lens AR (Choi et al., 2012).

Through its capacity to harm pancreatic cells, streptozotocin induces diabetes by causing hypoinsulinemia or hyperglycemia (Akbarzadeh et al., 2007). In our investigation, STZ caused metabolic parameters to change as well as a time-dependent rise in glucose and fall in insulin levels. The fasting blood glucose (FBG) levels of diabetic rats significantly decreased after receiving the extract continuously for 60 days. Similar activities have been reported for the curry leaves (El-Amin et al., 2013). Although the exact mechanism of the FBG-lowering action is yet unknown, the findings indicate that the methanol extracts function similarly to the common anti-diabetic medication glibenclamide by inducing surviving b-cells to produce

more insulin. This hypothesis has been supported by research showing that glibenclamide and a natural glucose - lowering product were successful in mildly diabetic rats but ineffective in severely diabetic group (Ivorra et al 1988; Sharma et al 1997).

An upsurge in the body's generation of free radicals leads to oxidative stress, which results in micro and macro vascular complications in diabetes. Based on the findings of this study, the extract is principal antioxidant that interact with free radicals and operate as free radical scavengers or blockers, which may prevent damage from free radicals in the body (Pandey et al., 2014). Oxidative stress and the development of diabetic complications including insulin resistance are closely related. The function and production of insulin are negatively impacted by a spike in oxidative stress, free fatty acid levels, FBG levels, and other factors (Jebur et al., 2016). Furthermore, MK extract reversed the damage caused by STZ in the diabetic mice by lowering the levels of serum parameters such as creatinine, HbA1C and BUN. The reduction in LDL and total cholesterol levels may be because of blockage of cholesterol pathway or stimulation of LDL receptors in hepatic cells (Verma et al., 2019). Altogether, restoration of lipid profile and serum parameters by methanolic extract point to reduced risk of developing diabetic comorbidities most notably CVD.

According to our findings, MK methanolic extract has a protective impact on the structure and activity of Langerhans cells and enhances the histological structure of the islet cells. The enhancement in the activity of the pancreas' enzymatic antioxidants, which are crucial to the defense system against free radical damage to the pancreas, may be the reason of the beneficial effect of the extracts (Abdul-Hamid et al., 2013).

5. Conclusion

These findings supported the traditional medical system's usage of curry tree seeds to treat DM and to prevent the diabetic complications. These results support *in-vitro* aldose reductase effect and antidiabetic effect further a formulation development and validation of Nutraceutical from MK seed will be fruitful. To clarify the precise mechanism of the hypoglycemic action of this plant, more thorough chemical and pharmacological studies are required.

References

1. Zheng, Y., Ley, S. & Hu, F. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. *Nat Rev Endocrinol* **14**, 88–98 (2018).
2. Ohta, M., Tanimoto, T., Tanakata, A., Hayakawa, T. (1993) Int. J. Biochem., Vol 25, 1165-1174.
3. Crabbe, M. J. C., Hoe, S.T. (1991) Enzyme, Vol 45, 188-193.
4. Varma, S. D., Kinoshita, J. H. (1976) Biochemical Pharmocology, Vol 25, 2505-2513.
5. Arulselvan P, Senthilkumar GP, Sathish Kumar D, Subramanian S. Anti-diabetic effect of Murraya koenigii leaves on streptozotocin induced diabetic rats. Die Pharmazie-An International Journal of Pharmaceutical Sciences.2006 Oct 1;61(10):874-7.

6. El-Amin M, Virk P, Elobeid MA, Almarhoon ZM, Hassan ZK, Omer SA, Merghani NM, Daghestani MH, Al-Olayan EM. Anti-diabetic effect of Murraya koenigii (L) and Olea europaea (L) leaf extracts on streptozotocin induced diabetic rats. Pak J Pharm Sci. 2013 Mar 1;26(2):359-65.
7. Jain, V., Momin, M. and Laddha, K., 2012. Murraya koenigii: an updated review. International Journal of Ayurvedic and Herbal Medicine, 2(04), pp.607-627.
8. Dineshkumar B, Mitra A, Mahadevappa M. Antidiabetic and hypolipidemic effects of mahanimbine carbazole alkaloid from Murraya koenigii Rutaceae leaves. International Journal of Phytomedicine 2010; 2:22-30.
9. Yadav, R. N. & Agarwala, M. (2011). Phytochemical analysis of some medicinal plants. Journal of Phytology, 3 (12), 2075-6240.
10. Brand-williams W, Cuvelier ME and Berset C.Use of free radical method to evaluate antioxidant activity .Lebensmittel Wissenschaft and Technologie 1995; 28(1): 25-30.
11. Chou HG, Kuo JT, Lin ES. Comparative antioxidant properties of water extracts from different parts of beefsteak plant (Perilla frutescens). J Food Drug Analysis. 2009; 17:489e496.
12. Elizabeth K, Rao MNA. Oxygen radical scavenging activity of curcumin. Int J Pharmaceut. 1990; 58:237–40.
13. Ebrahimzadeh MA, Nabavi SM, Nabavi SF, Eslami B, Rahmani Z. Antioxidant and antihaemolytic activities of the leaves of Kefe cumin (Lase trilobum L) Umbelliferae. Trop J Pharm Res. 2010; 9(5):441–9.
14. Haro-Vicente JF, Martinez-Gracia C, Ros G. Optimization of in vitro measurement of available iron from different fortificants in citric fruit juices. Food Chem. 2006; 98:639–48.
15. Oyaizu M. Studies on products of browning reactions: antioxidant activities of products of browning reaction prepared from glucose amine. Jap J Nutr. 1986; 44:307–15
16. Hayman S, Kinoshita JH. Isolation and properties of lens aldose. J Biol Chem 1965; 240: 877-882.
17. Jaslin E, Sethuramani A, Devi P, Meera R (2010). Antidiabetic, hypolipidemic and antioxidant activities of the plant extracts of Morus alba Linn. Int J Phytopharm Res 1: 25-28.
18. Cerelli MJ, Curtis DL, Dunn JP, Nelson PH, Peak TM, Waterbury LD (1986). Antiinflammatory and aldose reductase inhibitory activity of some tricyclic arylacetic acids. J Med Chem 29: 2347-2351.
19. Lowry, O. H., and Rosebrough, N. J., Farr, A. I., (1951) J. Biol. Chem., Vol 193, 265-275.
20. Termentzi A, Alexiou P, Demopoulos VJ, Kokkalou E. The aldose reductase inhibitory capacity of Sorbus domestica fruit extracts depends on their phenolic

content and may be useful for the control of diabetic complications. Die Pharmazie-An International Journal of Pharmaceutical Sciences. 2008 Sep 1;63(9):693-6.

21. Choi SJ, Kim JK, Jang JM, Lim SS. Inhibitory effect of the phenolic compounds from Geranium thunbergii on rat lens aldose reductase and galactitol formation. Korean Journal of Medicinal Crop Science. 2012;20(4):222-30.
22. Akbarzadeh A, Norouzian D, Mehrabi MR, Jamshidi SH, Farhangi A, Verdi AA, Mofidian SM, Rad B. Induction of diabetes by streptozotocin in rats. Indian Journal of Clinical Biochemistry. 2007 Sep;22(2):60-4.
23. Akter F, Rahman MM, Mostofa M, Chowdhury EH. Anti-diabetic effect of neem and spirulina in alloxan induced diabetic mice. Int J Current Res Acad Rev. 2014;2(4):124-34.
24. Ivorra, M. D., Paya, M., Villar, A. (1988) Hypoglycemic and insulin release effects of tormentic acid: a new hypoglycemic natural product. Planta Med. 54: 282 ± 285
25. Sharma, S. R., Dwivedi, S. K., Swarup, D. (1997) Hypoglycaemic, antihyperglycaemic and hypolipidemic activities of Caesalpinia bonducella seeds in rats. J. Ethnopharmacol. 58: 39 ± 44
26. Pandey G, Verma KK, Singh M. Evaluation of phytochemical, antibacterial and free radical scavenging properties of Azadirachta indica (neem) leaves. Int J Pharm Pharm Sci. 2014;6(2):444-7.
27. Jebur AB, Mokhamer MH, El-Demerdash FM. A review on oxidative stress and role of antioxidants in diabetes mellitus. Austin Endocrinol Diabetes Case Rep. 2016;1(1):1006-11.
28. Verma PK, Raina R, Sharma P, Sood S, Bhat MA. Attenuating potential of leaf extracts of alstonia scholaris on altered glycemic index, lipid, hepatic and renal parameters in streptozotocin induced diabetic rats. Journal Of Veterinary Pharmacology And Toxicology. 2019;18(2):39-48.
29. Abdul-Hamid M, Moustafa N. Protective effect of curcumin on histopathology and ultrastructure of pancreas in the alloxan treated rats for induction of diabetes. The Journal of Basic & Applied Zoology. 2013 Aug 1;66(4):169-79.