

## Antidiabetic effect of *Murraya koenigii* leaves aqueous extract on blood sugar levels in alloxanized diabetic rats

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### Abstract

**Introduction:** The present study was aimed to assess the antihyperglycaemic activity of *Murraya koenigii* leaves aqueous extract in alloxanized diabetic rats and to compare it with standard drug Metformin.

**Material and Methods:** Experimental diabetes was induced by intraperitoneal injection of alloxan to all groups apart from Group I treated as normal (negative) control. Group II served as diabetic (positive) control (distilled water only). Group III rats received *Murraya koenigii* aqueous extract (300 mg/kg, p. o) and Group IV rats received Metformin (150 mg/kg, p.o) for 28 days. Fasting blood sugar levels of rats were measured at the every week in the period of 28days.

**Results:** Oral administration of *Murraya koenigii* aqueous extract reduced the blood sugar levels significantly compared with the diabetic group.

**Conclusion:** The present findings of the study suggest that *Murraya koenigii* can be a promising antidiabetic agent which needs to be further explored.

**Keywords:** Alloxan, Biguanide related compounds, *Murraya koenigii*, Metformin, Diabetes

### Introduction

In past decades of history, many medicinal plants were used as antidiabetic agent with neither any scientific evidence nor proven their pharmacological reports. Six ethno botanically known plants having antidiabetic property like *Azadirachta indica*, *Murraya koenigii* (L.), *Ocimum tenuiflorum* (L.), *Syzygium cumini* (L.), *Linum itatissimum* (L.) and *Bougain villea spectabilis* have been pharmacologically well documented<sup>[1]</sup>.

Biguanide related compounds (BRCs) were evaluated quantitatively in many experimentally or clinically authenticated antidiabetic functional plant foods and potatoes. It was found in the results that the quantities of BRCs present in the chronological order: green curry leaves (*Murraya koenigii* (L.) Spreng) > fenugreek seeds (*Trigonella foenum-graecum* L.) > green bitter melon (*Momordica charantia* Descourt.) > potato (*Solanum tuberosum* L.). Garlic (*Allium sativum* L.) and sweet potato (*Ipomea batatas* (L.) found negligible amount of BRCs<sup>[2]</sup>.

*Murraya koenigii* Linn is belonging to family Rutaceae, commonly known as Meethi neem as folk name or Kadhipatta. It is an aromatic, more or less deciduous shrub or a small tree up to 6 m height found throughout India up to an altitude of 1500 m. Studies on phytochemistry of this plant have revealed the presence of carbazole alkaloids, volatile oil, glycozoline, xanthoxine and sesquiterpene<sup>[3]</sup>. *Murraya koenigii* is widely used as a spice for flavoring food and it may be a promising plant with minimal side effects.

Since adequate characterization of anti-diabetic activity of *Murraya koenigii* aqueous extract has not yet been done and considering that highest amount of BRCs was found to be present in *Murraya koenigii* leaves, an attempt was made to investigate the anti-hyperglycaemic activity of Aqueous extract of *Murraya koenigii* leaves and compare it with a standard biguanide i.e; Metformin in alloxan induced diabetic rats.

### Material and Methods

#### Drug and chemicals

Alloxan monohydrate (Loba Chemie, Mumbai, India), Metformin (Auro Pharmaceuticals, Mumbai, India), Blood glucometer (Bayer Healthcare, India) were procured. All reagents and chemicals used were of analytical grade and stored in a refrigerator at +4°C.

#### Collection and authentication of plant material

Leaves of *Murraya koenigii* were collected from the local area of Karad in Maharashtra, India (17.2760° N, 74.2003° E), certified

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and authenticated by Department of Botany, M. S. Shinde Mahavidyalaya, Tisangi, Kolhapur, India. The plant specimen voucher no: V03 (Ref: MHST/2016-17/28) of the plant was deposited in the herbarium. Fresh leaves were washed under tap water thoroughly; dried under shade and powdered by using a mechanical grinder.

#### Preparation of *Murraya koenigii* leaves aqueous extract

*Murraya koenigii* leaves aqueous extract was prepared by maceration method. About 200 g of leaf powder was subjected to cold maceration with chloroform:water in a conical flask for 7 days at room temperature. The flask was plugged with absorbent cotton at the mouth of flask and shaken periodically. It was filtered through a muslin cloth and the collected filtrate was re-filtered through Whatmann filter paper to get the clear filtrate. The filtrate was concentrated to dry residue by shade drying it for 30 days. This extract was labeled as AEMK and the selected dose was 300 mg/kg body weight (b w), per os (p.o)<sup>[4]</sup>.

#### Preparation of Metformin

Metformin solution was prepared by dissolving 150 mg of Metformin pure powder in 10 ml of distilled water to attain a concentration of 15 mg/ml, labeled as MET and its dose was selected as 150 mg/kg b w, p. o<sup>[5]</sup>.

#### Acute toxicity study of extract

AEMK was subjected to the acute toxicity test as per Organization for Environmental Control Development (OECD)-423 guidelines<sup>[6]</sup> for fixing a safe dose. A single dose of 2000 mg/kg b w of AEMK taken as a starting dose and all doses were orally administered to rats. LD<sub>50</sub> was determined and 1/10<sup>th</sup> of LD<sub>50</sub> was taken as therapeutic dose.

#### Experimental animals

Albino Wistar rats of either sex (weighing 100-250 g) were bred in the Central Animal House of the Krishna Institute of Medical Sciences, Karad. Animals were housed under standard conditions, maintained on a 12 hour light/dark cycle at 27-37°C in a diffusely illuminated room and had free access to food and water up to the time of experimentation. Animals were acclimatized to the laboratory environment before commencement of experiment.

The experimental protocol was approved by the Institutional Animal Ethics Committee. The care and use of laboratory animals were strictly in accordance with the guidelines prescribed by Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and the Indian National Science Academy Guidelines (INSA) for the use and care of experimental animals.

#### Inclusion criteria

Albino Wistar rats of either sex weighing 100-250 gm.

Albino Wistar rats with normal behaviour and activity

#### Exclusion criteria

Pregnant rats and those that have delivered once

Albino Wistar rats that were previously used for any other experimental purpose

#### Alloxan induced diabetes in rats

Alloxan was used to induce diabetes mellitus. Rats were fasted overnight. After overnight fasting, each rat was injected with freshly prepared 2% solution of alloxan monohydrate in 0.9% sodium chloride solution. The dose injected was 130 mg/kg body weight, intraperitoneally<sup>[7]</sup>. Rats with FBS > 150 mg/dl after 7 days of alloxan injection were selected for the study<sup>[7-9]</sup>. Diabetic rats were labeled as Alloxan induced diabetic (AID) rats while normal rats as non-diabetic (ND).

#### Experimental design

Albino Wistar rats of either sex were randomly divided into four groups (six animals per group) and they had free access to water and animal diet throughout the study period. Oral administration of Metformin and AEMK were given with appropriate doses at once daily at fixed time for the period of 28 days.

**Group I-NC:** ND rats treated with DW-5 ml/kg/day, b w, p. o served as normal control

**Group II-DC:** AID rats treated with DW-5 ml/kg/day, b w, p. o served as diabetic control

**Group III-AEMK:** AID rats treated AEMK-300 mg/kg/day, b w, p. o served as treatment group

**Group IV-MET:** AID rats treated with MET-150 mg/kg/day, b w, p. o served as standard group

#### Monitoring of blood sugar level during treatment

Blood samples of rats were collected by cutting tip of tails and checked fasting blood sugar (FBS) using blood glucose test strips with glucometer at day 0 and then every week interval of the study period. All animals were sacrificed by cervical decapitation at the end of study.

#### Statistical analysis

Results expressed as mean and standard error of mean (SEM). Data analyzed using one way analysis of variance (ANOVA) test and repeated measures ANOVA with *post hoc* Tukey Kramer's multiple comparison test. Significance considered when P value is less than 0.05. Statistical analysis was one using GraphPad InStat Software Inc, 1142E1, Camino Real 215, San Diego 9213, USA.

#### Results

Figure 1 represented FBS values of ND rats (n=6) during the study period as NC (negative control) group. The mean FBS of ND rats during observation in different periods (Day 0 to Day 28) varied from 76.33 mg/dl to 85.67 mg/dl. FBS values of ND rats observed during the entire study period were similar with no significant variation. (P = 0.0667) shown in Table 1.

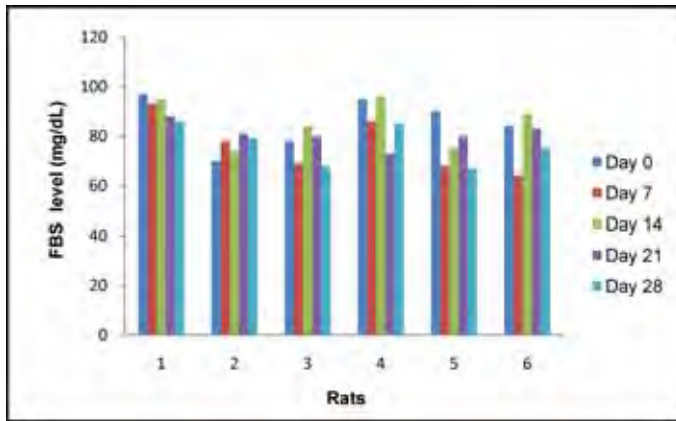


Fig. 1: FBS levels of ND rats with DW (5 ml/kg, b w, p. o)

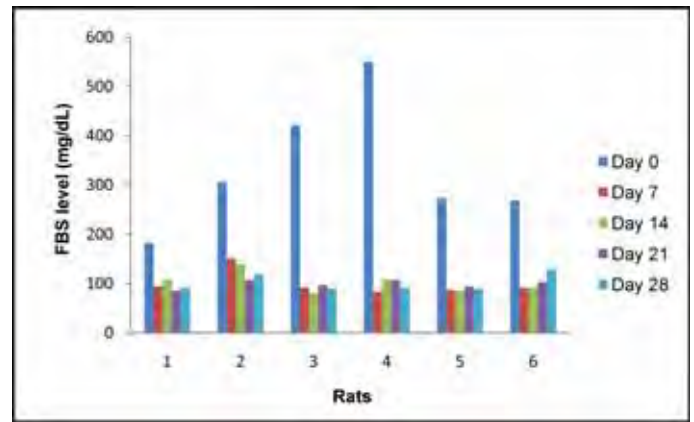


Fig. 3: FBS levels of AID rats treated with AEMK (300 mg/kg, b w, p. o)

Figure 2 depicted FBS values of AID rats treated with DW which served as DC (positive control) group. The FBS values of all the rats in this group remained consistently above 150 mg/dl (hyperglycaemic) with the mean FBS varying between 383 mg/dl to 400.83 mg/dl. The variation in the mean FBS values among the diabetic rats of this group (n=6) was not significant ( $P > 0.05$ ) shown in Table 1.

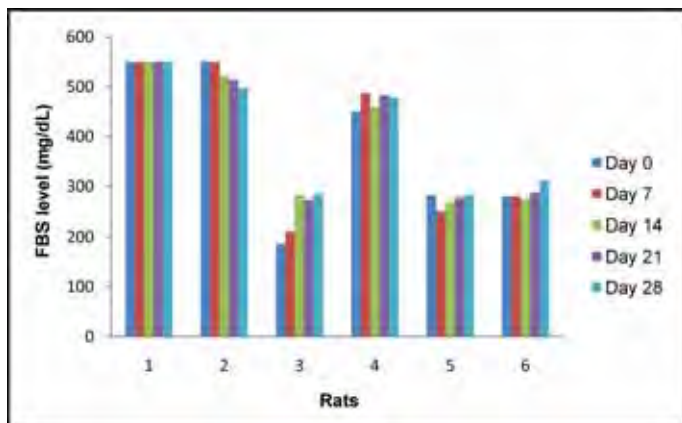


Fig. 2: FBS levels of AID rats with DW (5 ml/kg, b w, p. o)

Figure 3 showed the FBS values of AID rats treated with AEMK (300 mg/kg, p.o) served as treatment group. Day 0 represents the FBS values of AID rats prior to initiation of AEMK treatment. Day 7, 14, 21 and 28 represents the FBS values of AID rats after 7, 14, 21 and 28 days of treatment with AEMK (300 mg/kg, p.o) respectively. Pre-treatment mean FBS value (Day 0) was 333.5 mg/dl while post-treatment mean FBS value (from Day 7 to Day 28) varied between 98.83 mg/dl to 102.16 mg/dl. The above findings clearly shows that AEMK (300 mg/kg, p.o) significantly reduced FBS levels in AID rats and reduction in FBS levels were maintained throughout the study period during which the extract was given. Repeated measures ANOVA revealed that the variation in mean FBS value in this group was extremely significant ( $P < 0.0001$ ) shown in Table 1.

Figure 4 displayed the FBS values of diabetic rats treated with MET (150 mg/kg, p.o) served as standard group. Day 0 represents the FBS values of diabetic rats prior to initiation of MET treatment. Day 7, 14, 21 and 28 represents the FBS values of diabetic rats after 7, 14, 21 and 28 days of treatment with MET (150 mg/kg, p.o) respectively. Pre-treatment mean FBS value (Day 0) was 350.5 mg/dl whereas post-treatment mean FBS value (from Day 7 to Day 28) varied between 77.83 mg/dl to 148 mg/dl. Repeated measures ANOVA revealed that the variation in mean FBS value in this group was extremely significant ( $P < 0.0001$ ) shown in Table 1.

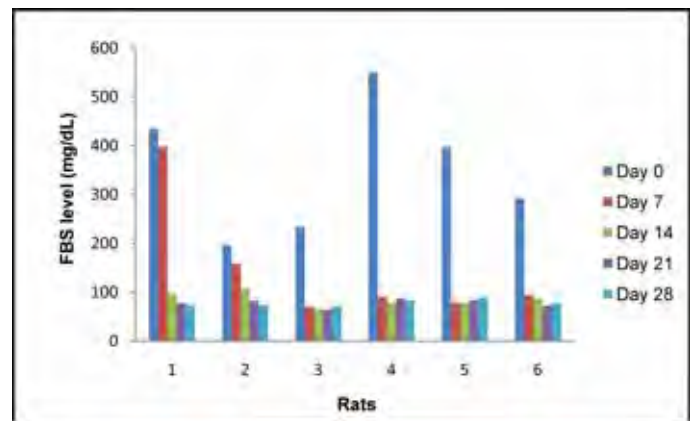


Fig. 4: FBS levels of AID rats treated with MET (150 mg/kg, b w, p.o)

Figure 5 displayed the mean FBS values of all groups. Day 0 represents the FBS values of diabetic rats prior to initiation of treatments. Day 7, 14, 21 and 28 represents the FBS values of diabetic rats after 7, 14, 21 and 28 days of treatment respectively. Pre-treatment mean FBS value (Day 0) was varied from each post-treatment mean FBS value (from Day 7 to Day 28). Repeated measures ANOVA revealed that the variation in mean FBS value in this group was extremely significant ( $P < 0.0001$ ) shown in Table 1.

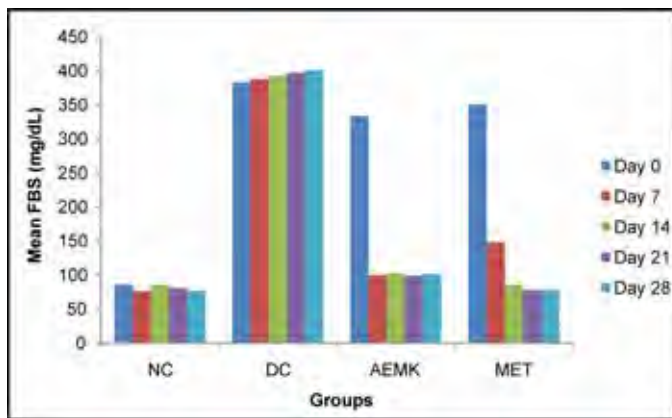


Fig. 5: Mean FBS levels of NC, DC, AEMK and MET groups

Table 1 summarized the mean FBS values of NC, DC, AEMK and MET groups observed throughout the study period (Day 0 to Day 28). Statistical analysis (One way ANOVA) showed that the variation in mean FBS values of groups was very significant ( $P < 0.01$ ) on Day 0 and was extremely significant ( $P < 0.001$ ) on Day 7, 14, 21 and 28. Inter-group analysis of Day 0, 7, 14, 21 and 28 was carried out (since one way ANOVA showed significant variation) using Tukey-Kramer’s multiple comparison test. Intergroup comparison between Group III and IV was not statistically significant. This indicated that the reduction in FBS due to Metformin and AEMK was comparable at all test intervals.

Table 1: Effect of *Murraya koenigii* aqueous extract on FBS in alloxanized diabetic rats

Day	0	7	14	21	28	F	P
Group	FBS measured in mg/dL						
I-NC	085.67 ± 04.24	076.33 ± 04.65	085.50 ± 03.90	080.83 ± 1.99	076.67 ± 03.33	2.606	0.066 <sup>NS</sup>
II-DC	383.00 ± 63.16 <sup>a**</sup>	388.00 ± 64.37 <sup>a***</sup>	393.16 ± 54.06 <sup>a***</sup>	397.33 ± 53.65 <sup>a***</sup>	400.83 ± 49.32 <sup>a***</sup>	0.517	0.723
III-AEMK	333.50 ± 53.55 <sup>a*</sup>	099.66 ± 10.39 <sup>b***</sup>	102.16 ± 08.70 <sup>b***</sup>	098.83 ± 03.50 <sup>b***</sup>	101.50 ± 07.24 <sup>b***</sup>	17.508	< 0.0001 <sup>***</sup>
IV-MET	350.50 ± 54.11 <sup>a**</sup>	148.00 ± 51.38 <sup>b**</sup>	085.83 ± 06.12 <sup>b**</sup>	077.83 ± 03.48 <sup>b**</sup>	078.00 ± 02.69 <sup>b**</sup>	13.005	< 0.0001 <sup>***</sup>
F	07.557	11.853	29.973	33.489	39.953	One way ANOVA	
p	0.0014 <sup>**</sup>	0.0001 <sup>***</sup>	< 0.0001 <sup>***</sup>	< 0.0001 <sup>***</sup>	< 0.0001 <sup>***</sup>		

Values expressed in Mean ± SEM, n= 6 number of rats, NS = Not significant,  $p < 0.01$  = very significant,  $p < 0.001$  = extremely significant, Tukey-Kramer multiple comparison test: a- Compared with Group I-normal control, b- Compared with Group II-diabetic control

### Discussion

Diabetes is one of the leading causes of morbidity and mortality throughout the world. It is a chronic disorder of carbohydrate, fat, and protein metabolism. Currently, six classes of orally effective anti-diabetic agents are used for the diabetes management including sulfonylureas, biguanides, thiazolidinediones, alpha-glucosidase inhibitors, fast-acting insulin secretagogues and DPP-4 inhibitors [10]. Beneficial effects of all these agents are well-documented but owing to several disadvantages. Successful diabetes management remains obscure even with a wide variety of therapeutics accessible to clinical practitioners [11]. A pure pharmacological approach of therapy to reverse the effects of diabetes is insufficient so an appropriate nutritional management is essential for restoring and maintaining a normal metabolic state.

Our findings of the present study were in agreement with the studies of Yadav et al. (2002) [12], Vinuthan et al. (2004) [13], and

Kesari et al. (2005) [14], Tembhrne et al. (2017) [15], Sarje et al. (2016) [16], Chaturvedi et al. (2014) [17], Vijayanand et al. (2015) [18] Singh et al. (2012) [19], Tembhrne et al. (2009) [20], and Bhopal et al. (2016) [21]. They have reported the antihyperglycaemic effect of *Murraya koenigii* leaves in terms of FBS only. It shows the similar hypoglycemic effect in spite of different solvents used for extraction like chloroform [18, 21], ethanol [15, 19], and ethyl acetate [16] apart from aqueous extract. Different parts of plant *Murraya koenigii* like roots [19], fruit juice [20] which have shown to exert blood sugar lowering effect similar to that of leaves.

During the present study, *Murraya koenigii* extract could exert comparable effect to Metformin, which is contradictory to the results of Lawal et al. (2008) [22]. They reported that the hypoglycaemic effect of this plant extract was significantly less as compared to another antidiabetic drug, Chlorpropamide. This difference could be attributed to the fact that Chlorpropamide



can induce hypoglycaemia whereas Metformin does not. On contrary, the study by Arulsevan *et al.* (2006) [23] stated that ethanolic extract of *Murraya koenigii* showed more effect than glibenclamide. The study reported by Adebayo *et al.* (2004) [24] has invalidated the antidiabetic ethno-medical claim of *Murraya koenigii*. Our results clearly indicate that AEMK has significant antidiabetic effect.

Based on observation and results in the present study the exact mechanism of antihyperglycaemic effect of *Murraya koenigii* aqueous extract could not be explained. But we suggest that higher concentration of BRCs in *Murraya koenigii* might be responsible for this Metformin like effect. On account of previous and current reports, various mechanisms have been postulated here. *Murraya koenigii* induces the increased utilization of glucose either through enhancing hepatic glycogenesis or diminishing glycogenolysis/gluconeogenesis reported by Khan *et al.*, (1995) [25] and our previous recent report (2018) [26]. It leads to the release of insulin from the  $\beta$  cells of pancreas. This insulin secretagogue effect leads to hypoglycaemic effect [13], exerting protective effects by decreasing oxidative stress and rejuvenating pancreatic  $\beta$  cells damage [27] and inhibition of pancreatic  $\alpha$ -amylase in the lysis of dietary starch to glucose which prevents the rate of glucose entry into the blood stream from the intestine [28]. Thus, the antihyperglycaemic properties of *Murraya koenigii* leaves aqueous extract may be synergize to the above mentioned mechanisms.

## Conclusion

Aqueous extract of *Murraya koenigii* has shown promising results of antihyperglycaemic activity in alloxanized diabetic rats. However further extensive studies need to confirm this activity in different animal models as well as human trials. If proven in human trials, this can be used as adjuvant to the existing antidiabetic drugs.

## Conflict of interest

The authors declare that there are no conflicts of interest.

## Acknowledgement

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