

# Studies on *in vitro* Anti-Diabetic Activities of the Epigeic Earthworm, *Eisenia fetida* Extract

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## ABSTRACT

**Aim:** Diabetes is considered as one of the serious complications and disorder in recent years. So, the present work was undertaken to investigate the anti-diabetic activity using *Eisenia fetida* earthworm extract. **Materials and Methods:** The anti-diabetic activity was carried out by using different concentrations of earthworm, *Eisenia fetida* extract with two different methods, alpha-amylase inhibitory assay and glucose uptake assay by using standard drug Metformin and Metronidazole respectively. **Results:** Results revealed that the earthworm, *Eisenia fetida* extract exhibit anti-diabetic activity by inhibiting alpha-amylase activity and also by increasing the glucose uptake in the cells. Thus, earthworm *Eisenia fetida* extract may serve as an alternative anti-diabetic drug to prevent diabetic complications in near future.

**Keywords:** Alpha-amylase assay, Anti-diabetic activity, *Eisenia fetida* and Glucose uptake assay.

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## INTRODUCTION

*Diabetes mellitus* is a chronic metabolic disorder where the body fails either to produce or utilize adequate amount of insulin. According to WHO professional committee 1999, there are four types of diabetes known as Type 1 (Insulin dependent Diabetes mellitus), Type 2 (Non-insulin-dependent Diabetes mellitus), Gestational polygenic disorder and other specific types (monogenetic type). In type 1 diabetes, there is anomaly in insulin production whereas in type 2 diabetes there is subnormal production of insulin or the body is resistant to insulin.<sup>[1]</sup> Presence of polydipsia, polyuria, asthenia, polyphagia and weight loss are the most common symptoms of diabetes.<sup>[2]</sup> Prolonged diabetes can cause serious life-threatening complications.

According to Indian Council of Medicinal Research (ICMR), more than 101 million people in India are affected by diabetes. As of 2021 data, 537 million people

of aged 20-79 are suffering from diabetes in the world as reported by Indian Diabetes Federation, whereas, 77 million people over the age 18 are affected by type 2 diabetes and 25 million are pre-diabetic as reported by WHO.

The WHO in its reports of 2016 on diabetes states that there is lack of proper policy with respect to healthy lifestyles, preventive measures and treatment of diabetes. Change in life style and lack of proper health care are the main reasons for increase in diabetes rate.<sup>[1]</sup> Various types of medical treatments concerning to diabetes are available such as somatic cell mediated aid, inhibitor medical care, anti-inflammatory drug treatment, dietary management, endocrine delivery devices, oral hypoglycemic agents etc.<sup>[3]</sup> Most of the times, the modern drug fails to treat the diseases and also causes innumerable side effects and complications.<sup>[4,5]</sup> Uncontrolled diabetes leads to the onset of other diseases such as renal failure, hypertension, cardiovascular diseases, neurological disorders, etc.<sup>[6-11]</sup> Though, diabetes and its complications are properly treated by medications, diet, physical exercise, controlling lipid profile, body weight management etc.<sup>[12,13]</sup> Yet, there is in need of reasonable, cost effective drug or therapy to treat diabetes properly with modern medicines.<sup>[14]</sup>

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Thus, scientists are in search of other alternative medicines, which are easily available with low cost and also to preserve them longtime with minimum side effects.

A system of traditional medicine that is practicing since mid-second millennium BCE, which is native to India is termed as Ayurveda, widely used to treat various ailments.<sup>[15]</sup> Earthworms are also known for their therapeutic values, showcasing a diverse array of biological effects such as anti-pyretic, anti-inflammatory, anti-fungal, anti-oxidant, anti-cancer, detoxifying, anti-hypertensive, anti-spasmodic, anti-ulcer, anti-allergic, anti-microbial and diuretic property.<sup>[16-18]</sup> Use of earthworms in Ayurveda has helped in understanding importance of integrative medicine and revealed different biological mechanisms.<sup>[15]</sup> Use of different earthworm species extract is one of the promising remedy for various diseases including anti-diabetic activities. Hence, the present study was undertaken to evaluate the anti-diabetic property by using the epigeic earthworm, *Eisenia fetida* extract through *in vitro* studies.

## MATERIALS AND METHODS

### Collection and preparation of earthworm powder

The epigeic earthworm, *Eisenia fetida* were obtained from the stock culture maintained in cattle manure at Vermitechnology laboratory, Department of Zoology, Karnatak University, Dharwad. 30 sexually matured healthy earthworms were washed in water to remove surface debris and fed with moist tissue paper for about 24 hr to clean up their gut. The gut-cleared earthworms were again washed and kept in closed Petri plates for about 3-5 days in sunlight. Then, the dried earthworms were finely powdered by using mortar and pestle. The obtained brown colored fine powder is further used for preparation of earthworm extraction.

### Preparation of earthworm extract

The *Eisenia fetida* earthworm extract was prepared by using Lysis buffer and Acetone, through cold treatment. The obtained residue was taken as earthworm extract.

### *In vitro* anti-diabetic assay

#### Alpha-amylase inhibitory assay

The alpha-amylase inhibitory assay was conducted following the protocol outlined by Ranilla *et al.*<sup>[19]</sup> with slight modifications by using *Eisenia fetida* earthworm extract as test sample. Here Metformin served as standard drug for this assay.

### Glucose uptake assay

Glucose uptake assay was studied by using yeast cells (*Saccharomyces cerevisiae*) cells as per the method prescribed by Cirillo *et al.*<sup>[20]</sup> Here, Metronidazole was used as standard drug for this assay.

### Calculations

The calculation method was employed to determine the percentage of alpha-amylase inhibition:

$$\text{Percent inhibition of alpha amylase (\%)} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{(\text{Absorbance of control})} \times 100$$

The increase in glucose uptake by yeast cells was determined by utilizing the following formula:

$$\text{Increase in Glucose uptake (\%)} = \frac{(\text{Absorbance of sample} - \text{Absorbance of control})}{\text{Absorbance of sample}} \times 100$$

## RESULTS

### Alpha-amylase inhibitory assay

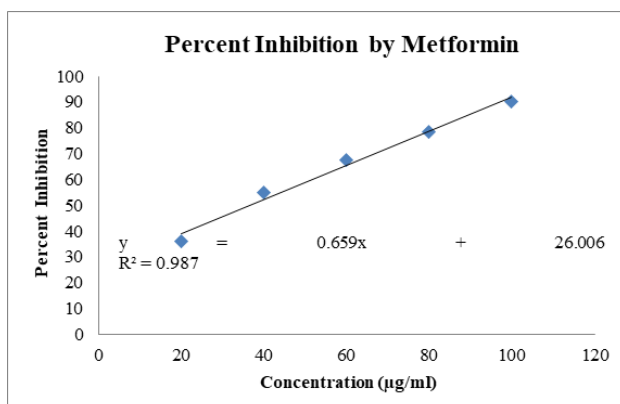
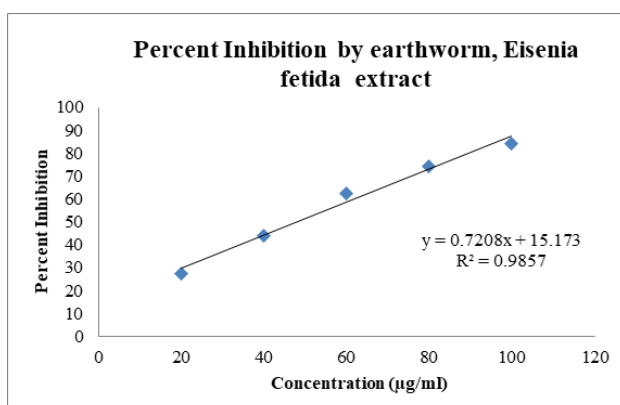
The earthworm, *Eisenia fetida* extract was subjected to alpha-amylase inhibitory assay with different concentrations (20, 40, 60, 80 and 100 µg/mL) by using Metformin as a standard drug. The results of percent inhibition and IC<sub>50</sub> value of Metformin and earthworm *Eisenia fetida* extract were tabulated and represented in Tables 1 and 2 and Figures 1 and 2, respectively. The standard drug, Metformin at 20, 40, 60, 80 and 100 µg/mL concentrations showed percent inhibition of 36.20%, 54.97%, 67.71%, 78.46% and 90.36%, respectively (Table 1 and Figure 1). Similarly, the extract derived from the *Eisenia fetida* earthworm has demonstrated the ability to inhibit alpha-amylase of 24.47%, 43.76%, 62.29%, 74.26% and 84.30% of percent inhibition at different concentrations of 20, 40, 60, 80 and 100 µg/mL respectively (Table 2 and Figure 2). The earthworm, *Eisenia fetida* extract showed highest percent inhibition of 84.30% at 100 µg/mL concentration whereas in standard drug Metformin it showed 90.36% inhibition at 100 µg/mL concentration. The standard drug comparatively showed more alpha-amylase inhibitory activity (IC<sub>50</sub>=36.40 µg/mL) than that of the earthworm, *Eisenia fetida* extract sample (IC<sub>50</sub>=48.31 µg/mL).

**Table 1: The data of alpha-amylase inhibitory assay and IC<sub>50</sub> value of the standard drug, Metformin.**

Sl. No.	Concentration (µg/mL)	O.D. at 540 nm	Percent inhibition (%)	IC <sub>50</sub> value (µg/mL)
1	20	0.5838	36.20	36.40 (µg/mL)
2	40	0.4120	54.97	
3	60	0.2954	67.71	
4	80	0.1970	78.46	
5	100	0.0882	90.36	

**Table 2: The data of alpha-amylase inhibitory assay and IC<sub>50</sub> value of the earthworm *Eisenia fetida* extract.**

Sl. No.	Concentration (µg/mL)	O.D. at 540 nm	Percent inhibition (%)	IC <sub>50</sub> value (µg/mL)
1	20	0.6636	27.47	48.31 (µg/mL)
2	40	0.5146	43.76	
3	60	0.3450	62.29	
4	80	0.2355	74.26	
5	100	0.1436	84.30	

**Figure 1: Percent inhibition of alpha-amylase activity by using the standard drug, Metformin.****Figure 2: Percent inhibition of alpha-amylase activity by using the earthworm, *Eisenia fetida* extract.**

## Glucose uptake assay

The earthworm extract underwent an *in vitro* glucose uptake assay utilizing yeast cells (*Saccharomyces cerevisiae*). Here, Metronidazole served as a standard drug. Results pertaining to glucose uptake assay were tabulated and represented in Tables 3 and 4 and Figures 3 and 4. Here, different concentrations like 50, 100, 150, 200 and 250 µg/mL of both standard drug and earthworm *Eisenia fetida* extract was subjected to glucose uptake assay for percent inhibition and IC<sub>50</sub> values. The standard drug, Metronidazole at different concentrations 50, 100, 150, 200 and 250 µg/mL showed percent inhibition of 31.25%, 50.25%, 62.52%, 72.95% and 85.73% respectively (Table 3 and Figure 3). Likewise, the earthworm, *Eisenia fetida* extract also shown percent inhibition of 12.51%, 28.32%, 44.44%, 60.76% and 76.64% at different concentrations of 50, 100, 150, 200 and 250 µg/mL respectively (Table 4 and Figure 4). The earthworm, *Eisenia fetida* extract showed highest percent glucose uptake of 76.64% at 250 µg/mL concentration, which was somewhat similar to 200 µg/mL concentration of standard drug, with 72.95%, whereas at 250 µg/mL concentration, the glucose uptake was 85.73%, which is far more than the earthworm, *Eisenia fetida* extract. The glucose uptake assay by yeast cells was better reported in the standard drug, Metronidazole with IC<sub>50</sub> value of 109.95 µg/mL than that of earthworm, *Eisenia fetida* extract with IC<sub>50</sub> value of 165.60 µg/mL.

**Table 3: The data of the glucose uptake assay and IC<sub>50</sub> value of the standard drug, Metronidazole.**

Sl. No.	Concentration (µg/mL)	O.D. at 540 nm	Percent inhibition (%)	IC <sub>50</sub> value (µg/mL)
1	50	0.3633	31.25	109.95 (µg/mL)
2	100	0.2639	50.25	
3	150	0.1973	62.52	
4	200	0.1443	72.95	
5	250	0.0744	85.73	

**Table 4: The data of the glucose uptake assay and IC<sub>50</sub> value of the earthworm, *Eisenia fetida* extract.**

Sl. No.	Concentration (µg/mL)	O.D. at 540 nm	Percent inhibition (%)	IC <sub>50</sub> value (µg/mL)
1	50	0.4624	12.51	165.60 (µg/mL)
2	100	0.3789	28.32	
3	150	0.2936	44.44	
4	200	0.2074	60.76	
5	250	0.1234	76.64	

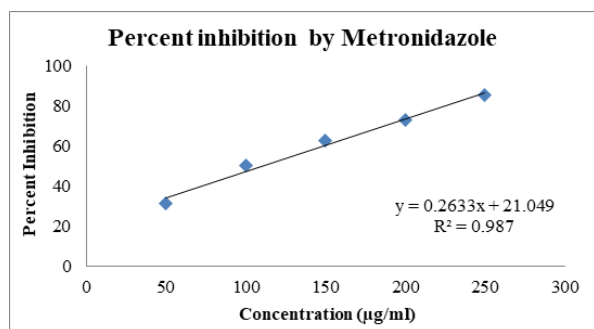


Figure 3: Percent inhibition of glucose uptake assay by standard drug, Metronidazole.

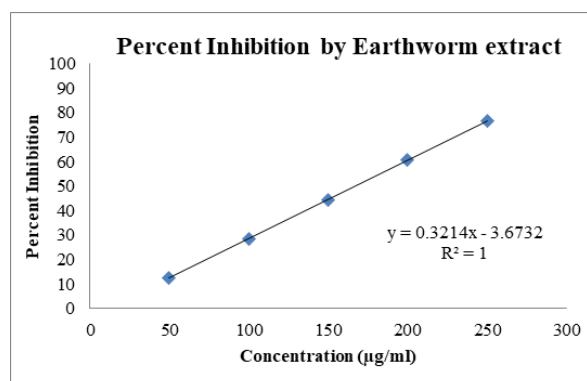


Figure 4: Percent inhibition of glucose uptake assay by using earthworm, *Eisenia fetida* extract.

## DISCUSSION

The use of herbal extracts to treat diabetes is familiar, but now days the use of animal extracts is gaining much importance. Earthworms are commonly used as medicines to treat various diseases and exhibit anti-inflammatory, anti-fungal, anti-oxidant, anti-cancer, detoxifying, anti-hypertensive, anti-spasmodic, antipyretic, anti-ulcer, anti-allergic, anti-microbial and diuretic properties.<sup>[16-18]</sup> Various zoo chemicals such as Alkaloids, Terpenoids, Saponin, Phlobatannin, Lignin and Coumarin were reported in *Eisenia fetida* earthworm extract which are responsible for these biological activities.<sup>[21]</sup> Anti-diabetic activity using *Eisenia fetida* earthworm extract was studied by two assays. Reports are also available on the anti-diabetic activity exhibited by the some varieties of earthworms. Amin<sup>[22]</sup> worked on the earthworm, *Pheretima asiatica* and studied anti-diabetic activity by inhibiting amylase production and alpha glucosidase enzyme, where the study found that the percentage of inhibition rose in correlation with the concentration of *Pheretima asiatica* extract, mirroring the findings of the current investigation. Soo<sup>[23]</sup> have studied *in vitro* anti-diabetic activity of earthworm *Lumbricus*

*rubellus* extracts by determining its ability to inhibit alpha-amylase activity by using Acarbose as standard drug and reported highest percent inhibition of alpha-amylase activity with IC<sub>50</sub> value 242.46 µg/mL with 95% ethanolic extracts whereas in the present study the standard drug, Metformin showed better results with IC<sub>50</sub> value 36.40 µg/mL and *Eisenia fetida* earthworm extract with IC<sub>50</sub> value 48.31 µg/mL therefore, it can be inferred that the anti-diabetic effects vary among different species and are concentration dependent. In the present study superior outcomes were noted with the standard drug however, the earthworm extract also demonstrates potential efficacy. Utilizing earthworm extract is advised due to its dual benefits in managing diabetes and its related complications. Numerous studies in the literature endorse the use of earthworm extract for diabetes treatment and its associated issues. Hany reported that the efficacy of earthworm extract in enhancing organ function which focused on the biochemical and histopathological changes in the liver, kidney, testis and pancreas of rats with experimentally induced diabetes mellitus type 1 using streptozotocin.<sup>[24]</sup> Wang (2023)<sup>[25]</sup> found that the earthworm extract PvE-3 demonstrates wound healing properties in cellular damage conditions associated with diabetes. Earthworm powder composite has been shown to have positive effects in the treatment of diabetic thrombosis through its ability to inhibit fibrinolysis activity, as well as its role in slowing down the progression of diabetes and diabetic nephropathy.<sup>[26]</sup> Thus, *Eisenia fetida* earthworm extract can be used to treat diabetes and its complications.

## CONCLUSION

The anti-diabetic assay was studied by using the earthworm, *Eisenia fetida* extract with two methods, i) alpha-amylase inhibitory assay and ii) Glucose uptake assay by using yeast cells. Results revealed that the standard drug, Metformin exhibited highest percent of alpha amylase inhibitory activity (90.36%) whereas, the earthworm, *Eisenia fetida* extract showed 84.30% inhibitory activity. Here, the standard drug, Metformin reported better alpha amylase inhibitory activity as IC<sub>50</sub> value is less 36.40 µg/mL than that of the earthworm, *Eisenia fetida* extract 48.31 µg/mL. Similarly, the standard drug, Metronidazole also exhibited the highest glucose uptake activity (85.73%) by yeast cells whereas, the earthworm, *Eisenia fetida* extract showed 76.64% at 250 µg/mL concentration. Here also the standard drug, Metronidazole shown better glucose uptake activity by

the yeast cells with  $IC_{50}$  value, is 109.95  $\mu\text{g}/\text{mL}$  but in *Eisenia fetida* earthworm extract  $IC_{50}$  value is more with 165.60  $\mu\text{g}/\text{mL}$ . Thus, the earthworm *Eisenia fetida* extract exhibit anti-diabetic activity by inhibiting alpha-amylase activity and also by increasing the glucose uptake in the cells.

Hence, it is clear that the earthworm, *Eisenia fetida* and their contents are having pharmaceutically important biomolecules which helps in treating diabetes. Thus, earthworm *Eisenia fetida* extract may serve as an alternative anti-diabetic drug to prevent diabetic complications in near future.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## SUMMARY

Diabetes is currently a prevalent disorder that necessitates more efficient medications. The medicinal properties of *Eisenia fetida* earthworm extract was investigated for its anti-diabetic potential through the examination of two crucial assays: alpha-amylase inhibitory assay and glucose uptake assay. The findings indicate that *Eisenia fetida* earthworm extract serve as a potent source for diabetes treatment due to their abundance of bioactive compounds which are having minimum or no side effects and also economically affordable.

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