



# Antidiabetic Activity of *Acmella Oleracea* Extracts Using In vitro And in Vivo Models

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### KEYWORDS

Acmella oleracea leaves, antidiabetic activity,  $\alpha$ -amylase,  $\alpha$ -glucosidase

### ABSTRACT:

Diabetes mellitus is one of the common metabolic disorders with micro-and macrovascular complications that results in significant morbidity and mortality. It is considered as one of the five leading causes of death in the world. In modern medicine no satisfactory effective therapy is still available to cure diabetes mellitus. There is increasing demand by patients to use natural products with antidiabetic activity due to side effects associated with the use of insulin and oral hypoglycemic agents. This present study evaluated the antidiabetic potential of the *Acmella oleracea* leaves extract. The antidiabetic potential was evaluated using STZ-induced diabetic rat model (in vivo) and inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase activity (in vitro). The ethanol extract of *Acmella oleracea* leaves exhibited a significantly ( $p < 0.05$ ) higher percentage reduction in fasting blood glucose and a concomitant increase in serum insulin level relative to the diabetic control group. The methanol extract also significantly reversed the alterations in oxidative stress markers occasioned by the diabetic condition. In conclusion, the result of the present study has demonstrated evidently that extracts of *Acmella oleracea* leaves ameliorates hyperglycemia and the associated oxidative stress in STZ-induced rats.

## Introduction

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and carbohydrate, protein and fat metabolism disturbances. Causes can be poor glycemia regulation of insulin secretion and/or diminished insulin signalling in key tissues (such as the liver) resulting in post-prandial or fasting hyperglycaemia. More evident symptoms are post-prandial sickness due to hyperglycaemia, increased food ingestion due to loss of appetite control, muscular proteolysis and adipose tissue lipolysis leading to severe body weight loss, glycosuria and osmotic polyuria, etc. Since causes can be spread between pancreatic, hepatic and peripheral tissues, combined treatments are a forthcoming choice: according to epidemiologic studies in USA, more than 30% of diagnosed diabetic patients are more than 30

years old and may receive insulin, 55% may receive another treatment [1]

There is a growing interest in natural resources as a source for developing nutraceuticals, drugs, pharmaceuticals, and various cosmetics. However, different natural resources used in traditional medicine are not supported with sufficient scientific information about their chemical constituents and biological properties [2-3]. In the recent years, many natural plant-based antioxidants either in the form of crude extract or functional foods are studied for their therapeutic potential in health management, such as diabetes or inflammatory processes. Scientific research supports the role of polyphenols in the prevention of diabetes mellitus and inflammatory processes. The bioactive compounds from extracts interact in a synergistic way,



and it is supposed to be advantageous in chronic, multifactorial diseases involving multiple pathways [4-6]. Recent studies have shown that inflammation and oxidative stress are closely associated with diabetes, but the involved mechanism is not clearly established because of the dual role of oxidative stress as a signal and as a damaging agent [7-8]

*Acmella oleracea*, also known as the toothache plant, has several traditional medicinal uses, most notably as an analgesic for toothache and other oral ailments due to its numbing effect. It is also used as a diuretic to help with bladder stones and is traditionally applied to treat conditions like rheumatism, snakebite, and sore throats. Modern research explores its antioxidant, anti-inflammatory, and potential anticancer properties [9-10]. This present study evaluated the antidiabetic potential of the *Acmella oleracea* leaves extract using STZ-induced diabetic Wistar rat model (in vivo) and inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase activity (in vitro).

## Material and methods

### Procurement and authentication of plant material

The leaves of *Acmella oleracea* were procured from local geographical area of Bhopal and authenticated by botanist. The leaves of *Acmella oleracea* was washed with water after dead cell scraping, chopped into small pieces for air-drying at room temperature for 7 days and coarsely powdered, stored in airtight containers and used for phytochemical and pharmacological studies.

### In Vitro Antidiabetic Assays

**Inhibition of  $\alpha$ -Amylase Activity:** The  $\alpha$ -amylase inhibition was measured according to Ranilla et al. [11] with a slight modification, as an anterior reported article. The sample solution  $\alpha$ -amylase from hog pancreas (0.5 mg/mL) was preincubated at 37 °C for 20 min. Then, starch solution (1%) was added, and incubation was achieved at the same temperature for 30 min. The reaction was stopped with dinitrosalicylic acid reagent. The absorbance was measured at 540 nm.

### $\alpha$ -Glucosidase Inhibition Assay

$\alpha$ -Glucosidase inhibitory activity was determined using the method described by McCue et al. [12] A mixture containing 50  $\mu$ L of sample solution, 125  $\mu$ L of  $\alpha$ -glucosidase from *Saccharomyces cerevisiae* (0.5 U/mL

in phosphate buffer solution, pH 6.9), and 700  $\mu$ L of phosphate buffer was preincubated at 37 °C for 15 min. After this time, 125  $\mu$ L of 5 mM p-nitrophenyl glucopyranoside was added, and the mixture was held for 30 min at 37 °C. The reaction was stopped by adding 1000  $\mu$ L of 0.2 M Na<sub>2</sub>CO<sub>3</sub>, and the absorbance was read at 405 nm. For the positive control in antidiabetic assays, acarbose was used. The  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory activities were expressed as a percentage of inhibition (IC<sub>50</sub>).

### In Vivo Antidiabetic activity

Studies will be carried out using Adult Wistar albino rats of either sex, weighing 200-250g respectively. The animals were housed in a group of 6 rats per cage under well-controlled conditions of temperature (22  $\pm$  2°C), humidity (55  $\pm$  5%) and 12hrs/12hrs light-dark cycle. Animals had free access to laboratory rat chow diet and R.O. water ad libitum. The protocol of the experiment approved by Institutional Animal Ethics Committee (IAEC) as per the guidance of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

### Acute Toxicity as per OECD guideline

Acute toxicity studies of ethanol extracts of *Acmella oleracea* was performed at dose levels of 100, 500 and 2000 mg/kg as per OECD guide lines. No mortality was observed in rats dosed with the ethanolic extracts at dose levels of 100, 500 and 2000 mg/kg (p.o). The treated animals did not demonstrate any significant changes in behavioral pattern and exhibited normal activity. Also there were no clinical signs of tremors, convulsions, exophthalmos, salivation, diarrhea and lethargy.

### Blood sample collection and analysis

Blood samples were collected by tail pricking method of each animal just after oral glucose administration at 0, 30, 60, 120 and 180 min for the estimation of glucose by accu-check glucometer.

### Antidiabetic activity of extracts

The animals were fasted overnight and diabetes was induced by a single intraperitoneal injection of freshly prepared STZ (60 mg/kg body weight) in 0.1 M citrate buffer-pH 4.5. After 72 hrs STZ-induced fasted rats having blood glucose levels greater than 250 mg/dl



were considered as diabetic and were used for further study. The animals had free access to 5% glucose solution overnight to overcome the drug induced hypoglycemia.

The experimental animals were divided into different groups of 6 rats in each group to determine the antidiabetic activity of ethanolic extracts of *Acmella oleracea* leaves. The actions of the extracts were compared with that of the standard oral hypoglycemic agent, glibenclamide. Dried extracts were redissolved in water using carboxymethyl cellulose (CMC) as suspending agent and this suspension was used for in vivo activity.

**Table 1: Experimental design for anti-diabetic study of *Acmella oleracea***

Group	Treatment
Normal Control	Control rats treated with normal saline
Diabetic Control	Diabetic control rats treated with normal saline.
Standard treated	Diabetic rats treated with Glibenclamide 5mg/kg b.w
EAAC 100 mg/kg	Diabetic rats treated with ethyl acetate extract of <i>Acmella oleracea</i> 100 mg/kg
EAAC 200 mg/kg	Diabetic rats treated with ethyl acetate extract of <i>Acmella oleracea</i> 200 mg/kg
MOAC 100 mg/kg	Diabetic rat treated with Methanol extract of <i>Acmella oleracea</i> 100 mg/kg
MOAC 200 mg/kg	Diabetic rat treated with Methanol extract of <i>Acmella oleracea</i> 200 mg/kg

After 72 hrs STZ-induced fasted rats having blood glucose levels greater than 250 mg/dl were considered as diabetic and were used for further study. The animals had free access to 5% glucose solution overnight to overcome the drug induced hypoglycemia. In study, overnight fasted experimental rats were different groups

of six rats (n=6) each to determine the antidiabetic activity of *Acmella oleracea* extracts. The diabetic groups of animals were administered with normal saline, standard drug (glibenclamide) and plant extracts for 28 days. The fasting blood glucose levels were estimated on the 0, 7th, 14th, 21st, and 28th day from the tail pricking of each animal. All animals ingested normal amounts of food and water during the study period; hence the body weight of all the rats were taken before and during the experimental protocol. At the end of the experimental period i.e., after 28 days, rats were sacrificed by cervical dislocation under mild anesthesia. Blood samples were collected through arterial Jugular with EDTA. Plasma and serum were separated by centrifugation at 3000 rpm for 10 min and the supernatant was transferred into labeled sample bottles and analyzed for various biochemical parameters associated with diabetes.

## RESULT AND DISCUSSION

### Macroscopic study

*Acmella oleracea* leaves are opposite, simple, and typically broadly ovate in shape, with a length of 5–11 cm and a width of 4–8 cm. The leaf margins are dentate (toothed), and the base is often truncate to shortly attenuate. The apex is acute to shortly acuminate, and the leaf surface is usually glabrous (hairless).

### Preliminary Phytochemical Screening

The qualitative phytochemical screening of the *Acmella oleracea* leaves for the presence of alkaloids, carbohydrate reducing sugars, glycosides like anthraquinones, flavanoids, saponins, tannins, phenolic compounds, fixed oils, fats, proteins, amino acids and sterols. Qualitative phytochemical screening of the various extracts of *Acmella oleracea* showed the presence of various secondary phytoconstituents. The methanolic extract was found to have a high content of alkaloids, flavonoids, saponins, and saponin glycosides. This shows that high polar secondary phytoconstituents were extracted with methanol when compared to petroleum ether.

### Determination of Total Phenolic content

The Total Phenolic content of plant extract was determined by using Folin–Ciocalteu reagent and Gallic acid as a calibration standard by using Gallic acid



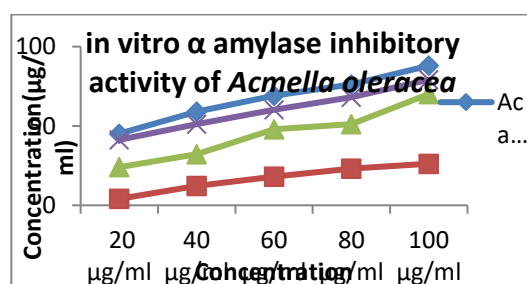
equivalence. The Gallic acid equivalence standard curve was plotted between concentration and absorbance. Total phenolic contents in plant extracts were obtained by using the regression curve equation. The total phenolic content was determined as gallic acid equivalent per mg of extract.

**Table 2: Total Phenolic Content of *Acmella oleracea* leaves extract**

S. No.	Extract	Total Phenolic content (mg GAE/g dry extract wt)
1	Petroleum ether	4.87 ± 0.32
2	Ethyl acetate	9.31 ± 0.25
3	Methanol extract	16.24 ± 0.72

**Table 3: *In vitro*  $\alpha$  amylase inhibitory activity of *Acmella oleracea* extract**

Concentration	20 $\mu$ g/ml	40 $\mu$ g/ml	60 $\mu$ g/ml	80 $\mu$ g/ml	100 $\mu$ g/ml
Acarbose	45.16	59.12	69.05	76.12	87.98
PEAC	4.08	12.13	18.03	23.14	26.21
EAAC	24.04	32.11	47.98	51.15	70.10
MOAC	41.25	51.17	60.32	68.07	79.14



**Figure 1: *In vitro*  $\alpha$  amylase inhibitory activity of *Acmella oleracea* extract**

**Table 4: *In vitro*  $\alpha$  Glucosidase inhibitory activity of *Acmella oleracea* extract**

Concentration	20 $\mu$ g/ml	40 $\mu$ g/ml	60 $\mu$ g/ml	80 $\mu$ g/ml	100 $\mu$ g/ml
Acarbose	39.27	49.61	61.29	73.01	83.24
PEAC	3.08	10.13	16.03	21.14	24.21

**GAE:** Gallic acid equivalence

***in-vitro* antidiabetic activity**

**Sample code for extract**

PEAC: Petroleum ether extract of *Acmella oleracea*

EAAC: ethyl acetate extract of *Acmella oleracea*

MOAC: Methanol extract of *Acmella oleracea*

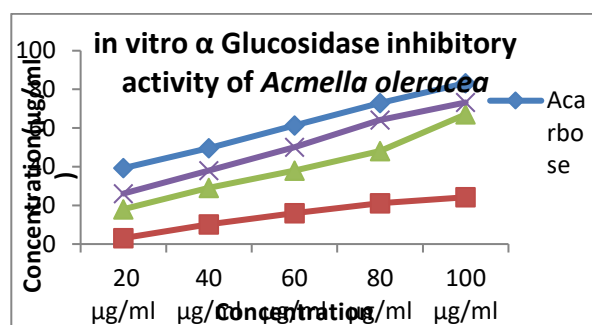
**$\alpha$ -Amylase inhibitory assay:**

The control of postprandial plasma glucose levels is important in the treatment of diabetes mellitus and its associated complications. Inhibition of enzymes involved in the metabolism of carbohydrates such as  $\alpha$ -amylase and  $\alpha$ -glucosidase, is an important therapeutic approach for reducing postprandial hyperglycemia.  $\alpha$ -Amylase found in the saliva and pancreatic juice, catalyses the hydrolysis of polysaccharides (such as starch) to disaccharides (like maltose and sucrose).

**$\alpha$ -Glucosidase inhibitory assay:**  $\alpha$ -Glucosidase inhibitory assay was carried out using a modified method by Adisakwattana et al. (2009).  $\alpha$ -Glucosidases are located in the mucosal brush border of the small intestine and catalyse the conversion of disaccharides to monosaccharide like glucose. In this study, methanol extract of *Acmella oleracea* exhibited the strongest inhibition of  $\alpha$ -glucosidase in dose dependent manner.



EAAC	18.04	29.11	37.98	48.15	67.10
MOAC	26.11	38.09	50.08	64.11	73.24



**Figure 2: in vitro  $\alpha$  Glucosidase inhibitory activity of *Acmella oleracea* extract**

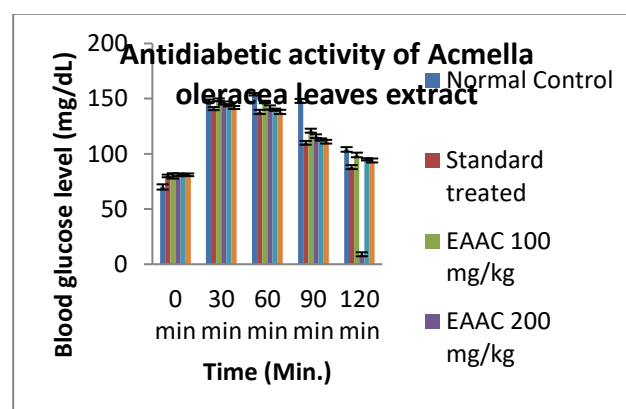
The result showed the inhibitory effects of different *Acmella oleracea* extract doses against  $\alpha$ -amylase and  $\alpha$ -glucosidase. In the  $\alpha$ -amylase assay, there were significant differences ( $p < 0.05$ ) in the percentage inhibition of the enzyme amongst all the concentration tested except at 20 and 40  $\mu\text{g/mL}$ . At all concentrations tested, there were significant differences ( $p < 0.05$ ) amongst all the extracts in the  $\alpha$ -glucosidase assay and  $\alpha$ -amylase. However at methanol extract of *Acmella oleracea* exhibited highest inhibition which is significantly to standard acarbose. Methanol extract of *Acmella oleracea* exhibited (49.63  $\mu\text{g/ml}$ ) IC<sub>50</sub> for the inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase (54.23  $\mu\text{g/ml}$ ) this is significantly comparable with ( $p < 0.05$ ) the standard (acarbose).

Herbal medicines are an economical and efficient approach for the management of type 2 diabetes by inhibiting key enzymes related to this disorder. The  $\alpha$ -amylase's and  $\alpha$ -glucosidase inhibitory activities of the crude extracts of *Acmella oleracea* showed a significant inhibitory activity against  $\alpha$ -amylase enzyme when compared to the positive control acarbose. Furthermore, the concentrated extracts of *Acmella oleracea* showed a significant  $\alpha$ -glucosidase inhibitory activity, as the standard. The studies showed that regular consumption of vegetables and specific tea in diabetic patients can lead to improved glycemic control, enhanced antioxidant defense system, attenuated oxidative stress, and inflammatory markers. Among the chemical constituents, polyphenols, as the main antioxidant

compounds, have demonstrated favorable effect in the management of diabetes mellitus as they regulate carbohydrate metabolism and stimulate insulin secretion but also affect fat metabolism.

#### Antidiabetic activity of extracts

The experimental animals were divided into different groups of 6 rats in each group to determine the antidiabetic activity of methanol extracts of *Acmella oleracea* leaves. The actions of the extracts were compared with that of the standard oral hypoglycaemic agent, glibenclamide.



**Figure 3: Effect of *Acmella oleracea* on the blood glucose levels in diabetic animals**

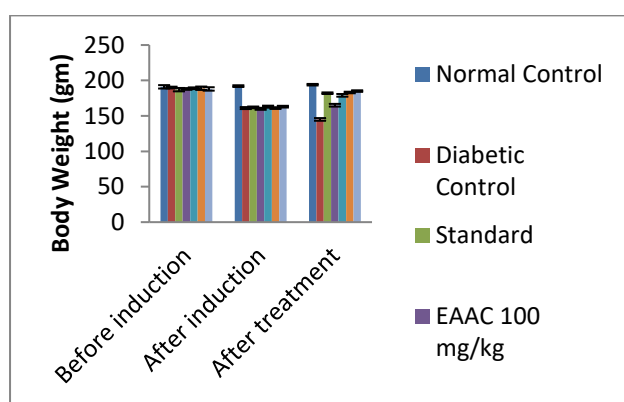
STZ induces diabetes similar to type II diabetes mellitus. STZ is a preferred agent to induce experimental diabetes since it has some advantages over alloxan such as, relatively longer half-life (15 min), sustained hyperglycemia for longer duration and the development of well characterized diabetic complications with fewer incidences of ketosis as well as mortality.

In study, it was found that fasting blood glucose level, which significantly elevated in STZ-induced diabetic rats by 205.72%, as compared to the normal control, got reduced on administration of CSE doses 100 and 200 mg/kg for 28 days. ethanol extracts of *Acmella oleracea* significantly  $p < 0.05$  reduced the blood glucose level toward normal as compared to diabetic control group.



### Effect of extracts on the body weight

All animals ingested normal amounts of food and water during the study period; hence the body weights of all the rats were taken before and during the experimental protocol. Slightly reduced body weight due to STZ induced diabetes, significantly reverted to normal in all animals except diabetic control group, whereas, in diabetic control group body weight reduced during 28<sup>th</sup> days, this was not observed with methanol extracts of *Acmella oleracea* treated.



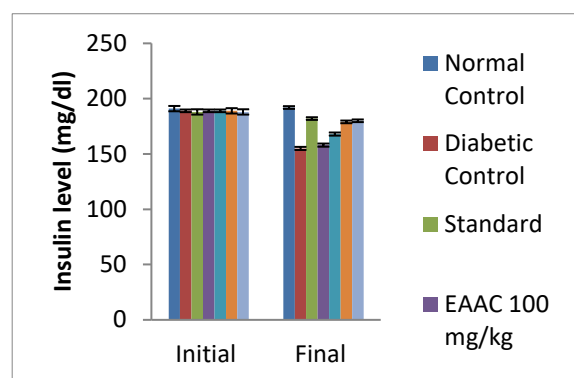
**Figure 4: Effect of *Acmella oleracea* on body weight of animals**

Body weights of all the diabetic rats were found to be statistically less ( $p < 0.001$ ) as compared to normal rats. All animals ingested normal amounts of food and water during the study period. Significantly reduced body weight of STZ induced diabetic rats as compared to normal control rats, significantly restored to normal in all animals except diabetic control group. Whereas, in diabetic control group body weight reduced during 28<sup>th</sup> days' diabetes study. The body weight of the diabetic rats treated with methanol extracts of *Acmella oleracea* at 100 mg/kg and 200 mg/kg were found to significantly improved. The percent decrease in diabetic control and increase treated groups in body weight on 28<sup>th</sup> day was indicating that the dose 200 mg/kg was more effective in controlling blood glucose levels and weight.

Induction of diabetes by STZ leads to loss of body weight due to the increased muscle wasting and loss of tissue proteins. The failure of diabetic animals to significantly gain weight during the course of time is due to continuous excretion of glucose because of the defect in peripheral uptake and impairment of liver's capacity to synthesize glycogen.

### Estimation of insulin level

After 28<sup>th</sup> day of treatment blood samples were withdrawn by in order to examine the insulin levels. Serum insulin was measured



**Figure 5: Effect of *Acmella oleracea* on insulin level**

The decrease in serum insulin level indicates hyperglycemia in STZ-induced diabetic rats. The STZ-induced diabetic rats exhibited maximum decrease in serum insulin levels on 28<sup>th</sup> day. The administration of *Acmella oleracea* extracts at different doses and Glibenclamide to diabetic rats for 28<sup>th</sup> day resulted in significant increase in serum insulin levels were observed.

### Conclusion

The use of ethno botanicals has a long folkloric history for the treatment of diabetic conditions however, it still suffers from extensive scientific validation. Therefore, the search for more effective and safer antidiabetic agents remains an important area of research. In this present study, the leaves of *Acmella oleracea* were evaluated for antidiabetic activity.

In light of the results, our study indicates that methanol extracts of *Acmella oleracea* have good antidiabetic activity. Methanol extracts of *Acmella oleracea* exhibited significant anti-hyperglycemic activities in STZ-induced hyperglycaemic rats without significant change in body weight; they can also improve the condition of Diabetic mellitus as indicated by parameters like body weight & lipid profile along with serum creatinine, serum urea and serum alkaline phosphatase.

Hence the above discussion reveals that methanol extract *Acmella oleracea* leaves at high dose (200 mg/kg) is



more effective and shows similar curative effect as standard that is, glibenclamide. This could be due to the possibility that some  $\beta$ -cells are still surviving to act upon by *Acmella oleracea* extract to exert its insulin releasing effect. Histopathological studies reinforce the healing of pancreas, by *Acmella oleracea* extracts, as a possible mechanism of their antidiabetic activity. In conclusion, the result of the present study has demonstrated evidently that extracts of *Acmella oleracea* leaves ameliorates hyperglycaemia in STZ-induced rats. This provides scientific evidence for the ethno medicinal use of *Acmella oleracea* and a baseline data for further work to characterize the extract.

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