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Research article

# *In-vivo* antidiabetic activity of *asparagus racemosus* seeds in streptozotocin induced diabetic model

**Anubhav Dubey*[1], Mamta Kumari[1], Vimal Kumar[2]**

[1] Department of Pharmacology, Maharana Pratap College of Pharmacy, Kanpur, Uttar Pradesh, India
[2] Department of Geography, Sai Meer PG College, Chhibramau, Uttar Pradesh, India

**Corresponding author:** Anubhav Dubey ✉ anubhavdwivedi803@gmail.com, **Orcid Id**: https://orcid.org/0000-0002-9089-4794



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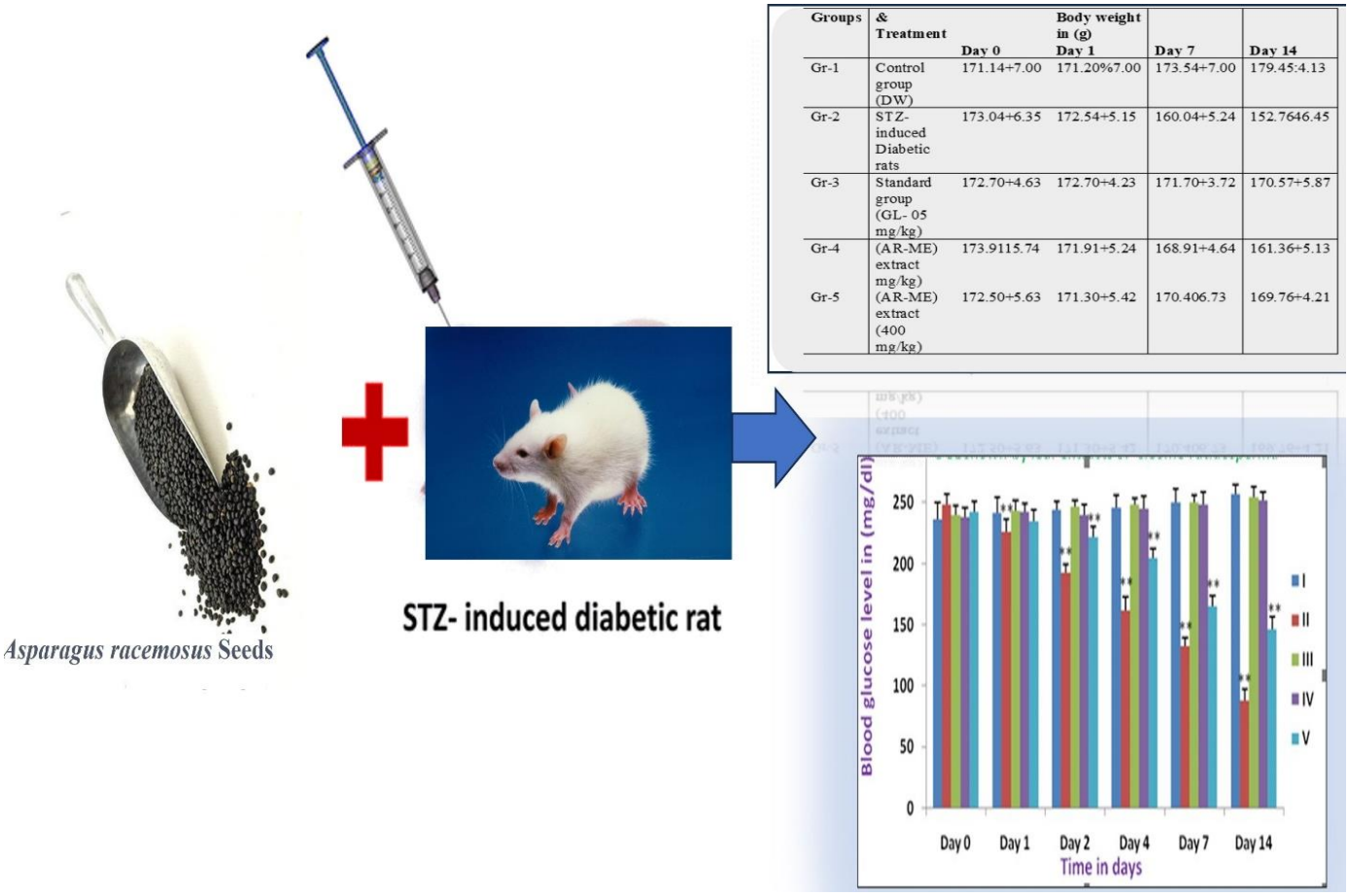
## ABSTRACT

The point of this study was to find out how well the methanolic seed extract of Asparagus racemosus treated diabetes in rats that had been given streptozotocin (STZ). We administered a single intraperitoneal injection of STZ (65 mg/kg) to the experimental animal to achieve the desired effect. We divided a group of adult male Wister albino rats into five groups: normal control, diabetic control, diabetic glibenclamide (5 mg/kg), diabetic methanolic seed extract of Asparagus racemosus (200 mg/kg), and diabetic methanolic seed extract of Asparagus racemosus extract (400 mg/kg).




| Groups | & Treatment | Body weight in (g) Day 0 | Day 1 | Day 7 | Day 14 |
|---|---|---|---|---|---|
| Gr-1 | Control group (DW) | 171.14+7.00 | 171.20%7.00 | 173.54+7.00 | 179.45:4.13 |
| Gr-2 | STZ-induced Diabetic rats | 173.04+6.35 | 172.54+5.15 | 160.04+5.24 | 152.7646.45 |
| Gr-3 | Standard group (GL- 05 mg/kg) | 172.70+4.63 | 172.70+4.23 | 171.70+3.72 | 170.57+5.87 |
| Gr-4 | (AR-ME) extract mg/kg) | 173.9115.74 | 171.91+5.24 | 168.91+4.64 | 161.36+5.13 |
| Gr-5 | (AR-ME) extract (400 mg/kg) | 172.50+5.63 | 171.30+5.42 | 170.406.73 | 169.76+4.21 |

We observed these rats for 14 days to measure their body weight (BW) and blood glucose levels. The STZ-treated diabetic rats showed a significant increase in blood glucose levels and a concurrent decrease in body weight. The study involved administering methanolic seed extracts of Asparagus racemosus (at doses of 200 and 400 mg/kg) and glibenclamide (at a dose of 5 mg/kg) orally to rats for a period of 14 days. The results indicated a substantial decrease in blood glucose levels and an increase in body weight compared to both the control group and the group treated with glibenclamide. The study revealed that the seed component of the Asparagus racemosus methanolic extract had a strong antidiabetic effect

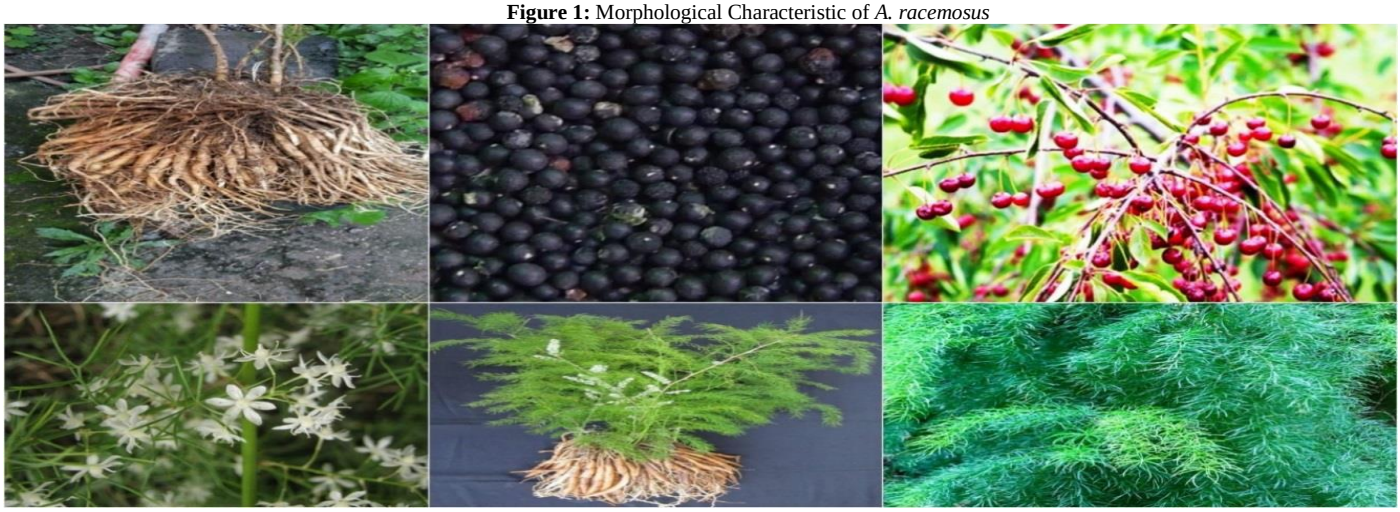
**Keywords:** Diabetes mellitus, Methaolic extract, Glibenclamide, Blood glucose, Streptozotocin.

## INTRODUCTION

The most common metabolic illness, diabetes mellitus, disrupts glucose homeostasis and alters carbohydrate, lipid, and protein metabolism as a result of insulin secretion or action abnormalities. The World Health Organisation projected diabetes as one of the main, potentially lethal illnesses by the year 2000, and it currently ranks among the top causes of morbidity and mortality globally. In terms of the global death rate, diabetes mellitus ranks sixth. Many different plants and plant extracts have shown promise in helping diabetics. Scientists propose that the majority of these plants exhibit hypoglycemic characteristics. To fight the complications of diabetes, scientists are looking for a new class of chemicals. Conventional herbal treatments for diabetes have only employed a small subset of the plants thought to have therapeutic effects. Most diabetics taking biguanides or other traditional anti-diabetic drugs today experience side symptoms such as headache, nausea, vomiting, stomach discomfort, diarrhoea, and vomiting. Countering these drug adverse effects requires the development of a novel herbal anti-diabetic composition that is both safe and effective [1–2].

One of the most important plants in Ayurveda is *asparagus racemosus*, sometimes called shatavari, since it may treat or prevent a wide variety of illnesses. It is the most powerful plant in the world, hence the name "herb's queen." Vitamins, alkaloids (racemosol), saponins (especially Shatavarins I, II, III, and IV), polyphenols, flavonoids, and steroidal glycosides are among its bioactive constituents. Ayurvedic and traditional medicine make extensive use of shatavari because of its sapogenin, which is a precursor to several pharmacologically active steroids. All parts of the plant have medicinal uses, but the most essential ones are the roots, stems, and leaves. An effective means of protection against disease are the "Rasayanas," crafted from shatavari. People use it to cure several disorders due to its phytochemical content. The phytochemicals found in it can alleviate many different diseases and ailments. Shatavari has numerous medicinal uses, including as an antispasmodic, antioxidant, anti-diabetic, anti-allergic, anti-malarial, preservative, anti-neoplastic, immune-modulatory, anti-arthritic, anti-inflammatory, anti-periodic, anti-ulcerogenic, antistress, anti-diarrheal, antidepressant, infection, tuberculosis, and many more. There are medications available that include Shatavari extract, and research has demonstrated that they can alleviate pain, fever, infection, abortion, and leprosy. The shatavari plant's roots, leaves, flowers, and stems have chemicals that can help with dyspepsia, depression, bronchitis, the throat, and the reproductive system [3–5].

**Figure 1:** Morphological Characteristic of *A. racemosus*



## MATERIAL AND METHODS

### Plant material and authentication of plant

Vatika Agro Shop in Jaipur, Rajasthan, India, 302020, is where we got the *A. racemosus* seeds. A renowned botanist confirmed the authenticity of the plant in question. The herbarium house of Janta Postgraduate College, A.P.S. University, is located at Rewa (486001), M.P., India. We extracted and dried a voucher specimen of the plant's seeds (J/Bot/2022APS-019) using a separate oven at 45 degrees Celsius. The mechanical grinder ground the dried seeds into a fine powder. A Soxhlet used a Soxhlet apparatus to extract 52 g of seed powder from 500 mL of solvent ethanol and water. Ducted normal-pressure evaporation in a water bath to concentrate the extracted filtrate.

### Making the plant extract

We heated 500 g of coarse powder in a Soxhlet apparatus, then extracted it using a solvent system that included petroleum ether (40–60 oC), chloroform (CL), methanol (ME), and purified water (AQ) for 24 hours each. We gathered the samples and allowed them to dry at room temperature. We then packed and preserved the final product.

### Chemicals and medications

Kanpur is where we obtain our sodium chloride, D-glucose, methanol, citric acid monohydrate, sodium citrate tribasic dehydrate, STZ, and glibenclamide.

### Research subjects and data collection methods

The study used Wister albino rats, both male and female, weighing 150–200 g. We housed the rats in an animal facility with good ventilation, subjecting them to a 12-hour light/dark cycle and maintaining a constant temperature and relative humidity of 55–60%. Large polypropylene cages with rice husks as bedding housed the rats. Unless it was fasting time, the animals could eat and drink everything they wanted. We replaced the bedding twice a week and tagged each cage with an identifying label. We applied picric acid to each rat's head and/or tail to identify each cage individually.

### Experimental procedure

### Phytochemical screening

The methanolic extract underwent qualitative testing to detect and identify numerous constituents such as tannins, alkaloids, saponins, glycosides, terpenes, phenolics, flavonoids, carbohydrates, proteins, and steroids. We conducted the identification of these components using well-established and uncomplicated qualitative procedures, as described by Trease and Evans (Table 1) [3-4].

### Evaluation of toxicity

The Organisation for Economic Co-operation and Development (OECD) (423) set the recommendations for the evaluation of acute toxicity [13]. The study selected adult albino mice in optimal physical condition with a weight range of 20 to 25 g. prior to administration, the three we deprived the three animals of food overnight before administering it, but we kept water available. With traditional herbal treatment, it was highly unlikely for death to occur at the highest starting dosage of 2000 mg/kg body weight (BW). Therefore, we conducted a limit test on all animals, administering a single dosage of 2000 mg/kg BW. Each animal was individually identified. After the treatment, we observed each animal individually for the first 30 minutes, and then frequently throughout the first 24 hours, paying particular attention to We then examined the animals for the next 72 hours to look for any signs of mortality. After the initial clinical tests, we conducted additional evaluations and documented the results [7].

### *In vivo* anti-diabetic effect

As part of an in vivo study, researchers investigated the potential antidiabetic effects of a methanolic seed extract from *Asparagus racemosus* in diabetic Wistar rats. We used an intraperitoneal injection of 50 mg/kg BW of STZ to induce diabetes. There was a comparison between the usual medicine, glibenclamide, and the antidiabetic impact of plant extract [8-13].

### Induction of diabetes in rats

After a week of acclimatization, we fasted the rats overnight to induce diabetes. We dissolved the STZ in a citrate buffer pH 4.5, a mixture of two parts 0.1 M sodium citrate and three parts 0.1 M citric acid, and then injected it intraperitoneally to induce diabetes. To prevent drug-induced hypoglycemia due to excessive insulin release from cells, we gave the animals a 5% glucose solution to drink overnight. The study enrolled animals as diabetics if their blood glucose levels exceeded 250 mg/dl after three days of measurement. The four-day milestone after STZ injection marked the beginning of the fourteen-day treatment period, during which the plant extract was administered. [14].

### Experimental design

All animals had their starting weights and fasting glucose levels recorded when the trial began. All participants in the study had their blood glucose levels checked on a regular basis with an (on call plus glucometer).

We created each group of six rats from the thirty rats used in the studies.

**Gr 1:** Normal control rats received distilled water.

**Gr 2:** STZ-induced diabetic rats received (from 1st day) distilled water and served as diabetic control for 1-14 days.

**Gr 3:** STZ-induced diabetic rats received standard drug glibenclamide (5 mg/kg) for 1-14 days.

**Gr 4:** STZ-induced diabetic rats received the plant seed extract (200 mg/kg BW) for 1-14 days.

**Gr 5:** STZ-induced diabetic rats received the plan seed extract (400 mg/kg BW) for 1-14 days.

BW was assessed in all rats prior to the onset of diabetes and on the 4th, 7th, and 14th days of treatment. Blood glucose levels were determined on the 1st, 7th, and 14th days through tail tip cutting. Upon completion of the experiment on the 14th day, an adequate amount of blood was obtained via retro-orbital bleeding from all animals while under anesthesia for the evaluation of biochemical parameters (blood glucose and BW) [15].

**Evaluation using statistical methods**

We used ANOVA to analyse all the parameters after Dunnett's test [16]. We used the mean and SD to represent the outcomes. We performed the statistical analysis using software versions 6.0 of Graph and Prism [17].

## RESULTS

**Analysing plant compounds**

The phytochemical study of the (AR) extracts identified alkaloids, glycosides, saponins, flavonoids, steroids/triterpenoids, tannins/polyphenolics, carbohydrates/reducing sugars, and amino acids/proteins.

**Acute toxicity study**

An acute toxicity investigation revealed the chemical to be non-toxic at a dose of 2000 mg/kg. There was no evidence of toxicity or harmful effects, such as tremors or abnormal motor activity, even after 14 days. Therefore, we determined the therapeutic dosage for future pharmacological research to be 200 mg/kg, which is one tenth of the dose [17].

***In vivo* anti-diabetic effect**

Table 2 displays the results about the effects of glibenclamide (5 mg/kg), *Asparagus racemosus* methanolic seed extract (200 mg/kg and 400 mg/kg), and other substances on diabetic rats. When comparing the first and fourteenth days, the average body weight decreased in grades 2, 3, 4, and 5. The body weight of the vehicle control animals remained rather constant, but the rats who were given diabetes had a notable decrease, with a range of 172.54±5.15 g on the first day to 152.76±6.45 g on the fourteenth. A substantial decrease in body weight (BW) was seen in Gr 2 (diabetic rats) compared to the other groups ($p<0.05$). When 200 µg kg of methanolic seed extract of *Asparagus racemosus* and 5 mg/kg of glibenclamide were given to diabetic rats, their body weight (BW) went from 173.91±5.74 g on the first day to 161.36±5.13 g on the 14th day. This was after the rats had lost weight due to diabetes. In the same way, giving 400 µg kg of methanolic seed extract of *Asparagus racemosus* to animals that had been given glibenclamide (5 mg/kg) reversed the weight loss from 173.70±4.63 g on the first day to 169.76±4.21 g on the 14th day. Table 3 displays the effects of glibenclamide (5 mg/kg), CR leaf extracts (200 mg/kg and 400 mg/kg), and diabetic rats. The rats were induced with diabetes using a methanolic seed extract of AR. Diabetic rats (Gr 2) significantly elevated their blood glucose levels. This decrease in fasting blood glucose levels continues to rise until the third week comes to a close. Figure 3 shows that there was a statistically significant decrease in blood glucose levels ($p<0.05$) when 400 mg of the extract was used compared to 200 mg.

## DISCUSSION

Phytochemical screening is a way to evaluate the plant's quality. Coloured responses can demonstrate the presence or absence of bioactive substances. Medicinal plants include phytoconstituents that have curative effects against a variety of human ailments. The biological evaluation of two concentrations of the plant's *Asparagus racemosus* seed extract (200 mg/kg BW) and extract (400 mg/kg BW) was prompted by the discovery of several active phytoconstituents in the methanol extract, but not carbohydrates. In plant extractions, methanol is a useful solvent since it is non-toxic, evaporates easily at low temperatures, acts as a preservative, and cannot cause the extract to complex or dissociate. Because of this, it is reasonable to assume that the phytochemicals included in plant extracts may help ward off diabetes mellitus and other metabolic diseases by neutralising free radicals. Because of their lower toxicity, natural compounds have shown promise as new medicines for treating a variety of diseases. The fatal dose not only determines the extract's poisonous level but also helps determine the effective dosage for the experiment. At all of the dosages tested, the animals in this study showed no signs of toxicity or death throughout the duration of the trial.

According to acute toxicological investigations, oral administration of AR methanolic seed extract up to 2000 mg/kg BW was well-tolerated and did not result in death. There was a notable disparity in body weight ($p<0.05$) between the 200 mg/kg and 400 mg/kg dosages. The methanolic seed extract of AR showed an improvement in BW, indicating its dose-dependent antidiabetic potential. When compared to diabetic rats, the study found that the methanolic extracts of the treatment groups caused a decrease in blood glucose levels. The extracts may improve glucose utilisation and metabolism. Compared to healthy control rats (Gr 1), the STZ-induced diabetic rats (Gr 2) in this study exhibited a substantial increase in blood glucose levels. In contrast, after 14 days of treatment with methanolic extracts plus the conventional medication glibenclamide, diabetic rats showed a reduction in blood glucose level. Methanolic seed extracts abolished these effects in diabetic rats. The results suggest that AR's methanolic seed extract might mitigate diabetes-related BW problems and coronary risk factors.

**Table 1:** Results of phytochemical screening of *Asparagus racemosus*

| Test for chemical groups | Pet. Ether -extract | Chloroform - extract | Methanol - extract | Aqueous - extract |
|---|---|---|---|---|
| Alkaloids. | -- | + | + | + |
| Glycosides. | -- | -- | + | + |
| Saponins | -- | -- | + | + |
| Flavonoids. | -- | -- | + | + |
| Steroids / Triterpenoids | + | -- | + | -- |
| Tannins/Polyphenolics | -- | -- | + | + |
| Carbohydrate/Reducing sugars | -- | -- | -- | + |
| Amino acids/Proteins | -- | -- | + | + |
| *(+) denotes present; (--) denotes absent | | | | |

**Table 2:** Effect of methanolic seed extract of the of *Asparagus racemosus* on body weight in Streptozotocin-induced diabetic rats.

| **Groups** | **Treatment** | | **Body weight in (g)** | | |
|---|---|---|---|---|---|
| | | **Day 0** | **Day 1** | **Day 7** | **Day 14** |
| Gr-1 | Control group (DW) | 171.14+7.00 | 171.20%7.00 | 173.54+7.00 | 179.45:4.13 |
| Gr-2 | STZ-induced Diabetic rats | 173.04+6.35 | 172.54+5.15 | 160.04+5.24 | 152.7646.45 |
| Gr-3 | Standard group (GL- 05 mg/kg) | 172.70+4.63 | 172.70+4.23 | 171.70+3.72 | 170.57+5.87 |
| Gr-4 | (AR-ME) extract mg/kg) | 173.9115.74 | 171.91+5.24 | 168.91+4.64 | 161.36+5.13 |
| Gr-5 | (AR-ME) extract (400 mg/kg) | 172.50+5.63 | 171.30+5.42 | 170.406.73 | 169.76+4.21 |

*Values expressed as mean±SD(n=6),Values are statistically significant at P<0. 05 using one-way Anova fallowed by Dunnetts test. Methanolic seed extract of the of Asparagus racemosus, DW: -Distilled water,STZ-Streptozotocin,

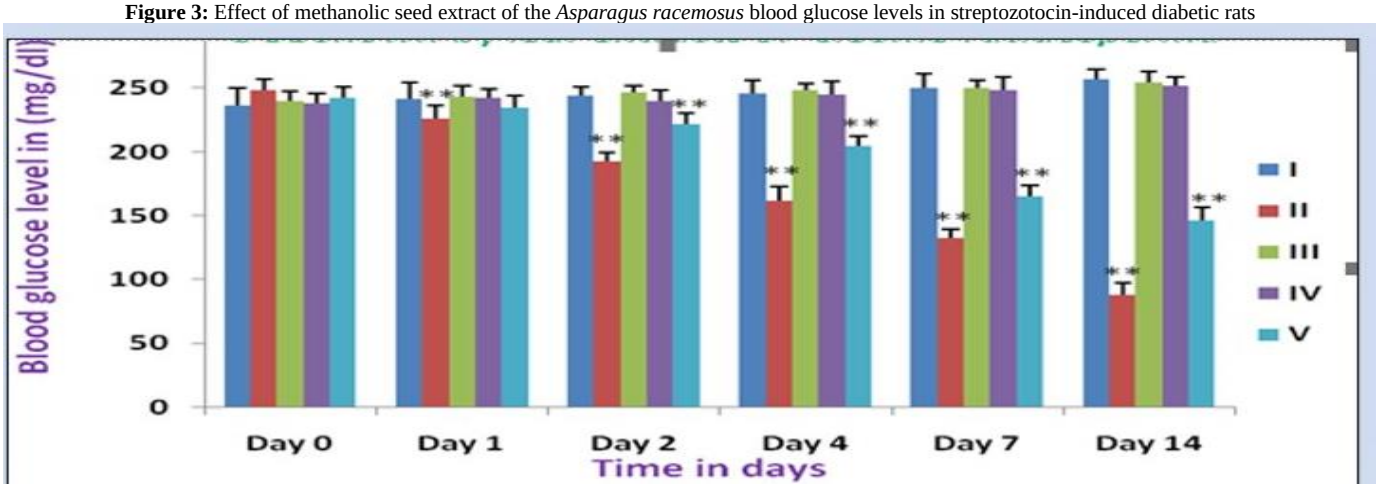
**Figure 2:** Determination of blood glucose level

**Table 3:** Effect of different extracts of methanolic seed extract of the of *Asparagus racemosus on blood glucose levels in streptozotocin-induced diabetic rats.*

| Groups & Treatment | BGL (mg/dl) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Day 0 | Day 1 | Day 2 | Day 4 | Day 7 | Day 14 | F value |
| I | 236.25 ±13.55 | 241.83 ±12.18 | 244.33 ±6.40 | 246.00 ±10.00 | 249.87 ±11.17 | 256.85 ±7.55 | 0.54 |
| II | 248.03 ±9.17 | 226.35 ±10.25** | 192.66 ±7.21** | 162 10.76** | 132.5 ±7.12** | 87.66 ±9.30** | 0.42 |
| III | 240 ±7.34 | 243.53 ±8.64 | 246.5 ±5.30 | 248.15 ±5.57 | 249.85 ±6.40 | 254.73 ±8.15 | 0.90 |
| IV | 238 ±8.14 | 242.5 ±6.72 | 240.19 ±7.83 | 245 ±10.32 | 248.5 ±9.77 | 251.47 ±7.36 | 0.71 |
| V | 242.15 ±9.10 | 234.88 ±9.04 | 221.36 ±9.26** | 204.5 ±7.81** | 165.16 ±8.28** | 146.55 ±10.09** | 0.85 |

*Values expressed as mean±SD(n=6).The data were statistically analysed by one-way ANOVA, fallowed by Dunnets t-test.p values less than 0.05 were considered significant.*p<0.05;p,0.01.Figure in parenthesis indicates %fall in BGL as compared to 0 day.

**Figure 3:** Effect of methanolic seed extract of the *Asparagus racemosus* blood glucose levels in streptozotocin-induced diabetic rats




## CONCLUSION

Glucose estimation aided in the diagnosis and progression of diabetes mellitus. The typical range for fasting blood glucose in healthy individuals is 70 to 100 mg/dL. An insulin shortage primarily causes hyperglycemia, a common complication of diabetes, when this level rises to 500 mg/dL or more. The methanolic extract significantly reduced blood glucose levels after 14 days of continuous therapy. This plant was shown to dramatically lower the glucose level in diabetic rats caused by STZ ($p<0.05$). The study demonstrated a dose-dependent impact, with the action of AR becoming evident at lower doses. The results of these early phytochemical investigations provide promise for a new line of anti-hyperglycaemic medications by suggesting that AR seeds have positive effects on diabetes mellitus. This task can benefit further experimental analysis in the future.

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**Author Contributions:** All authors equally participated.

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