International Journal of Research in Medical Sciences
*Devi LJ et al. Int J Res Med Sci. 2026 Mar;14(3):992-995*
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: https://dx.doi.org/10.18203/2320-6012.ijrms20260615

**Original Research Article**

# A study on the antihyperglycemic effect of aqueous extract of *Sida rhombifolia* leaves on blood glucose level of experimental animals

**Laitonjam Jonita Devi[1]*, Indira Raleng[2], Laishram Elizabeth Devi[1], Oinam Joychandra Singh[1]**

[1]Department of Pharmacology, Shija Academy of Health Sciences, Imphal, Manipur, India
[2]Department of Pharmacology, Jawaharlal Nehru Institute of Medical Sciences, Imphal, Manipur, India

**Received:** 04 October 2025
**Revised:** 11 November 2025
**Accepted:** 18 February 2026

***Correspondence:**
Dr. Laitonjam Jonita Devi,
E-mail: jonitalaitonjam777@gmail.com



## ABSTRACT

**Background:** Medicinal plants are used for the treatment of many ailments. *Sida rhombifolia* which has various medicinal properties is investigated for its effects on blood glucose level of the experimental animals.
**Methods:** The cleaned dried leaves of *Sida rhombifolia* was Soxhlet with water for *Sida rhombifolia* extract (SRE). 53 albino rats were recruited. Acute toxicity test was done by administering up to the dose of 2000 mg/kg to 5 albino rats but no toxicity was observed. The SRE at the doses of 200 mg/kg, 400 mg/kg were administered orally at 0 hour, ½ hour, 1 hour and 2 hours to 24 normal rats. Similarly, the standard drug metformin (0.5 mg/kg) was also administered orally. The blood glucose levels (BGL) of the rats were estimated. Then the same doses of SRE and metformin were administered orally to 24 diabetic induced rats at the same point of time. The BGLs of the diabetic rats were estimated. The MBGLs of the diabetic rats after administration of the extract and standard were compared to the MBGLs of the diabetic control.
**Results:** The effect of the extract of BGLs of the diabetic rats were found to be statistically significant. But the observation on the comparison of the MBGLs of the standard and diabetic control was insignificant. Since the SRE contains phytochemicals like flavonoids, its probable mechanism of action is increasing of sensitivity of insulin or act as an insulin secretagogue.
**Conclusions:** Therefore, SRE may have antihyperglycemic property.

**Keywords:** Antihyperglycaemic, Albino rats, Blood glucose level, Metformin, Streptozotocin, *Sida rhombifolia* extract

## INTRODUCTION

Traditional healers use many herbs for the treatment of different ailments. The awareness of the usage of medicinal plants has also been increased among the physicians and pharmacists.

These plants have gained a good place for the management of chronic diseases- diabetes, CNS disorders and different types of cancers. Further there is the emergence of reverse pharmacology in the research Lab of the MNC. Among the herb the local traditional healers frequently used *Sida rhombifolia*for the treatment of diabetes, gastritis, indigestion etc.[1,2] It is also found to have anticancer properties.[3] Therefore, the present study was conducted for the hypoglycemic as well as antihyperglycemic properties of *Sida rhombifolia* (malvaceous) (in local name known as Uhan in Manipuri).

### *Aims and objective*

The effect of aqueous extract of *Sida rhombifolia*on-blood glucose level in normal rats, blood glucose level in streptozotocin induced diabetic rats.

## METHODS

It is a preclinical experimental study which was conducted in the Post Graduate research laboratory, department of pharmacology, Jawaharlal Nehru Institute of medical Sciences, Imphal, Manipur for a period of 2 years from August 2019 to August 2021. The protocol of the study was approved by the Academic society of JNIMS and IAEC of RIMS (Reg. no 1596/GOI/12 CPCSEA dated 12/12/2019).

Fresh leaves of *Sida rhombifolia* was collected and identified by the Life Science Department, Manipur University (authentication no L.S D M U- 000244). The leaves were cleaned with water and air dried in the shade for several days. The aqueous extraction of *Sida rhombifolia* leaves (SRE) was done with some modification (Verma SCL and Agarwal SL4). The test drug (SRE), Standard (Metformin) were given orally. Selection of drug doses: the test drug-SRE- 200 mg/kg, 400 mg/kg, standard -metformin 300 mg/kg hyperglycaemic agent streptozotocin 50 mg/kg intraperitoneal.[6,7] The diabetic agent–Streptozotocin (STZ) 50 mg/kg intraperitoneally and standard drug- Metformin 0.5 mg/kg orally were selected.

### *Estimation of blood glucose level*

24 healthy rats were kept fasting for 18 hours with free access to water and care taken to prevent coprophagy. Drop of blood was collected from tail vein. And BGL was estimated by glucose oxidase method (Barham and Trinder P).[8] The rats were treated with SRE, metformin orally using intragastric feeding tube. The BGL of each rats were estimated at 0, 30, 60 and 120 mins respectively.

### *Induction of Streptozotocin*

The overnight fasted rats were treated with a single dose of STZ (IP)- 50 mg/kg. The rats were fed with 5% glucose solution for next 24 hours to prevent hypoglycemia. After 72 hours of STZ administration blood glucose level was estimated as before. Rats having BGL above 200 mg/dl were selected for the study. On the 7th day the rats were kept fasting for 18 hours, the BGL were estimated as before. Then, the rats were treated with SRE and metformin and BGL were estimated as before at 0, 30, 60 and 120 min.

### *Selection and grouping of animals*

53 healthy albino rats, 24 rats for normal study, 24 rats for hyperglycemia induced study, 5 rats for acute toxicity study of either sex weighing 120-200 gm, 5 months aged were recruited and kept in polypropylene cages for 10 days for acclimatization Lab atmosphere. The rats feds on balance diets and water ad libitum and care taken to avoid coprophagy. The animals were selected because of small size, low cost, omnivorous resemble to human being nutritionally

### *Toxicity testing*

Acute toxicity test of the extract was conducted to 5 rats by giving the extract orally up to the dose of 2000 mg/kg orally as per OECD/OCED guideline 425.[9]

### *Statistical analysis*

Descriptive statistics, mean, SD, SE, Anova, Dunnett t test.

## RESULTS

### *Hypoglycaemic activities of SRE*

*At 0 minutes*

Blood glucose level of dose1 and dose 2 were insignificantly lower when compared with those of the standard drug.

*At 30 minutes*

Blood glucose level of dose 1 and dose 2 were insignificantly lower when compared with that of standard drug.

*At 60 minutes*

Blood glucose level of dose 1 and dose 2 were insignificantly lower when compared with those of standard drug

*At 120 mins*

blood glucose level of dose 1 were insignificantly lower when compared with those of the standard drugs.

*At 0 minutes*

Blood glucose level of dose 1 and dose 2 were insignificantly lower when compared with those of standard drugs

*At 30 minutes*

Blood glucose level of dose 1 were insignificantly lower when compared with that of the standard drug.

*At 60 minutes*

Blood glucose level of dose 1 were insignificantly lower when compared with those of the standard. But blood glucose level of dose 2 was significantly lower (p=0.001) when compared with those of standard.

*At 120 minutes*

Mean blood glucose level of dose 1 was insignificantly (p=0.01) lower when compared with those of standard but

the mean blood glucose level of dose 2 were significantly lower (p=0.001) when compared with those of standard. In short, the insignificant effect of the dose 2 (SRE=400 mg/dl) on blood glucose level of the STZ induced hyperglycaemic albino rats at 30 min, 60 min was observed but at 120 min the significant effect of the same dose was observed when compared with the effect of the standard drug.

**Table 1: Effect of aqueous extract of *Sida rhombifolia* leaves on blood glucose level in normal albino rats (Mean+, n=6).**

| Groups | Drugs & dosage | Blood glucose levels (mg/dl) at | | | |
|---|---|---|---|---|---|
| | | Fasting | 30 mins | 60 mins | 120 mins |
| **Control** | Gum acacia 2% in D/W | 89±1.52 | 90.33±1.64 | 93.50 0.99 | 96.33±1.58 |
| **Test dose1** | SRE (200 mg/kg) in 2% gum acacia suspension. | 94.33±2.75 | 95.16 0.98 | 93.16 | 87.00±3.212 |
| **Test dose 2** | SRE (400 mg/kg) in 2% gum acacia suspension. | 95.5±1.05 | 94.50±1.02 | 91.66±1.35 | 87.66±1.94 |
| **Standard** | Metformin (300 mg/kg) | 91.66±1.74 | 88±1.69 | 84.33±2.20 | 80.83±3.03 |
| **One-way Anova** | F | 2.6 | 6.15 | 8.55 | 6.28 |
| | DF | 3,20 | 3,20 | 3,20 | 3,20 |
| | P | 0.07 | 0.004 | 0.001 | 0.004 |

*p<0.05 compared to control group

**Table 2: Effect of aqueous extract of *Sida rhombifolia* leaves blood on Streptozotocin induced hyperglycaemic albino rats.**

| Groups | Drugs & dosage | Blood glucose levels (mg/dl) at | | | |
|---|---|---|---|---|---|
| | | Fasting | 30 mins | 60 mins | 120 mins |
| **Diabetic control** | Gum acacia in 2% D/W | 227.00±2.39 | 238.00±1.93 | 248.50±1.60 | 258.67±1.45 |
| **Test dose 1 (mean sem)** | Test dose 1(200mg/kg) | 219.00±2.23 | 211.33±2.40 | 200.00±2.62 | 200.17±2.68 |
| **Test dose 2** | Test dose 2 (400 mg/kg) | 220.00±2.25 | 215±1.93# | 200.00±2.25 | 170.83±4.02# |
| **Standard** | Metformin (300 mg/kg) | 227.67±6.12 | 219.67±2.92 | 203.50±3.36 | 193.50±32 |
| **One-way Anova** | F | 772.42 | 703.07 | 593.4 | 472.56 |
| | DF | 2,25 | 2,25 | 2,25 | 4,25 |
| | P | 0.001 | 0.001 | 0.001 | 0.001 |

#p<0.05 as compared to standard group (by applying ANOVA, Dunnett T test)

## DISCUSSION

*Sida rhombifolia* is one of the herbs used by the traditional healer for many ailments diabetes and GIT diseases etc. The universal solvent water was used for the extraction medium so that any active ingredient of the leaves was not disturbed. Because of the omnivorous in nature and human being nutritionally, the albino rats was selected. Visible signs and symptoms of acute toxicity was observed

The hypoglycemic effect of the SRE was studied on the BGL of Normal albino rats but no significant result was observed in comparison to the effect of metformin on BGL of normal rats. The findings were almost conformed to the report of Das L et al.[12] This showed that the SRE does not possess the hypoglycemic activity on normal blood glucose level. Similarly, the antihyperglycemic effects of SRE was studied on BGL of the STZ induced hyperglycemic elevated blood sugar level of the albino rats. The effect of the aqueous extract of the *Sida rhombifolia* on the blood glucose level of the streptozotocin induced diabetic rats after administering with the dose 200 mg/kg, 400 mg/kg at 30 mins, 60 mins and 120 mins revealed the statistically significant lowering of the blood glucose level when compare to the blood glucose level of the diabetic control group treated with the standard metformin. This finding supports the findings of Prabhakar et al.[13] The lowering in blood glucose level was more significant with the dose of 400 mg/kg of *Sida rhombifolia* extraction at 30 mins but the effect was less than that of standard drug Metformin (300 mg).

Metformin at the dose of 300 mg/kg reduces the blood glucose level of Streptozotocin induced diabetic rats significantly from (227.67±6.1 mg/dl) to (193.50±3032 mg/dl) which is consistent with the findings of Ignacinmuthu et al and Amalraj et al whereas the extract pf *Sida rhombifolia* reduces the blood glucose level of STZ induced diabetic rats form (220.16 mg/dl to 170.83 mg/dl).[14] The finding conformed the findings to of Moukett et al.[15] The observation on the lowering of blood glucose level of the STZ induced diabetic rats may suggest that the extract may possess hypoglycemic activity in diabetic rats. The phytochemical study of the *Sida rhombifolia* which is executed by Anooj et al showed that the extract of the SR contains flavonoid, alkaloids, coumarin, tannins, terpenoids, glycosides, phytosteroids and anthraquinons.[16] Therefore the probable mode of

action of the aqueous extract of *Sida rhombifolia* causing the lowering of the blood glucose level is due to the presence pf the above mentioned phytochemicals which hamper the absorption of glucose and related polysaccharides. The standard drug metformin decreases the absorption of glucose, amino acids and Vitamin B 12 from the GIT. The present study highlights for a detail investigation of the different ingredients of the extract of the *Sida rhombifolia* for the different properties like lowering of blood glucose level.

***Limitation***

It is a simple preclinical experimental study. The phytochemicals and pharmacological evaluation is required to be carried out in order to identify the active principles responsible for antihyperglycaemic activity and its mechanism of action.

## CONCLUSION

SRE does not produce significant lowering on the BGL of the normal healthy albino rats and normal dose of the SRE does not produce significant effect on the BGL of the STZ induced hyperglycemic albino rats within 30 to 60 mins but the higher dose (400 mg/dl) of the SRE produces significant effect on the BGL of the STZ induced hyperglycemic suffering albino rat after 60 minutes and in 120 minutes. It is assured that SRE is not having hypoglycemic properties but antihyperglycemic properties (like standard metformin) antihyperglycemic agent with slow effect on BGL.

*Funding: No funding sources*
*Conflict of interest: None declared*
*Ethical approval: The study was approved by the Institutional Ethics Committee*

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**Cite this article as:** Devi LJ, Raleng I, Devi LE, Singh OJ. A study on the antihyperglycemic effect of aqueous extract of *Sida rhombifolia* leaves on blood glucose level of experimental animals. Int J Res Med Sci 2026;14:992-5.