Evaluation of antidiabetic potential of *Hibiscus rosa sinensis* on streptozotocin-induced diabetes on Wistar albino rats

K. Chauhan  
Department of Zoology, M. D. University, Rohtak (Haryana), India  
S. Rani*  
Department of Zoology, M. D. University, Rohtak (Haryana), India  
*Corresponding author. E-mail: sudeshrani.zoo@mdurohtak.ac.in

How to Cite

Abstract
The chronic metabolic disease known as diabetes mellitus causes hyperglycemia in the body. Antioxidant and antidiabetic qualities are well-known benefits of *Hibiscus rosa sinensis* (HRS). In this work, diabetic Wistar albino rats were used to assess the antidiabetic properties of HRS flower extract. A total of 18 animals were taken and divided into three groups (n = 6)– (Group 1): Normal control group, (Group 2): Diabetic control group, (Group 3): Diabetic group treated with a 125 mg/kg dose of HRS flower extract. Group 2 animals showed a progressive decrease in body weight, while Group 1 animals showed a considerable gain in body weight. After overcoming the weight loss, Group 3 animals also showed an increase in body weight that was similar to Group 1. Group 2 animals had blood glucose levels higher than 400 mg/dL, but Group 1 animals had blood glucose levels below 200 mg/dL throughout the experiment. Group 3 animals first had glucose levels higher than 350 mg/dL and then lower than 200 mg/dL, comparable to Group 1 animals. Upon histological examination, the pancreatic islets of Group 2 animals showed vacuolation, necrosis, and degeneration. The animals in Group 3 displayed regenerated islets of Langerhans and enhanced pancreatic anatomy. The animals in Group 3 also returned to normal in terms of body weight and blood glucose levels, similar to those in Group 1. These findings show that Hibiscus rosa sinensis has potential as an alternative diabetic treatment; further research is needed to fully understand its modes of action and long-term effects.

Keywords: Diabetes, Glucose, *Hibiscus rosa sinensis*, Histology, Pancreas

INTRODUCTION
Diabetes mellitus is a chronic disorder and is presented by high blood glucose levels (Budin et al., 2018). It is a prevalent metabolic condition that impacts diabetics' metabolism of fat, protein, and carbohydrates (Adyeyemi and Adewole, 2019). Diabetes mellitus can occur in two ways- 1) failure of insulin secretion due to β-cells destruction of the pancreas, which is known as Type I diabetes. 2) Failure of insulin action due to prolonged exposure to insulin is known as type II diabetes (Goycheva et al., 2006). It was estimated that in the year 2005, about 1.1 million deaths occurred because of diabetes, and mostly these people belong to developing countries. WHO states that up to the year 2030, the rate of these deaths will be doubled (Pethe et al., 2017). The International Diabetes Federation estimates that 463 million individuals worldwide were diagnosed with diabetes in 2019, and that number is projected to increase to 700 million by 2040 (Saeedi et al., 2019). Known as an autoimmune illness, type I diabetes mellitus is brought on by the death of pancreatic β cells, which leaves a shortage of insulin (Sabry et al., 2020). Due to this, the blood glucose level rises, which leads to several complications like retinopathy, neuropathy, nephropathy, and cardiomyopathy (Pillai and Mini, 2016). Streptozotocin (STZ) is used to induce diabetes in laboratory animals. STZ is a β-cytotoxin that selectively damages the pancreatic β cells and causes diabetes (Punithavathi et al., 2008). Several plants in traditional medicine are known to have antidiabetic properties. The genus *Hibiscus* is widely used due to its hypoglycaemic properties. Researchers have been focusing on several varieties of the genus Hibiscus to identify a treatment for diabetes mellitus in recent years (Afiune et al., 2017). Widely cultivated shrub *Hibiscus*
*H. rosa sinensis* (HRS) is known to have antidiabetic, antifungal, antibacterial, antioxidant, anticancer, cardioprotective, and anti-inflammatory properties (Missoum, 2018). *H. rosa sinensis* belongs to the Malvaceae family and is a perennial, woody, and ornamental plant (Venkatesan et al., 2021). It is typically found in tropical regions (Ansari et al., 2020). It is an extravagant flowering plant with different flower petal colours, such as red, white, yellow, orange, peach, pink, purple, etc (Viado et al., 2022). The red flower variety has been preferably used in medicine since ancient times (Al-Snafi, 2012). Plant-derived antioxidants help improve the oxidative stress caused by diabetes. The antioxidant qualities of *H. rosa sinensis* flower extract are well established (Zaki et al., 2017).

In India's rural population, *H. rosa sinensis* is used as a natural cure for diabetes. Previous studies also stated its antidiabetic activity in different laboratory animals (Afiune et al., 2017; Kumar et al., 2013). The glycemic level is reportedly lowered by the insulin-secreting action of crude HRS extract (Moqbel et al., 2011; Vimala et al., 2008). Extract of *H. rosa sinensis* significantly improves the structure of beta cells of the pancreas and increases the size, number and diameter of pancreatic islets in diabetic albino rats (Pethe et al., 2017). Therefore, reducing the glycemic level. It was capable of preserving the normal structure of Langerhans islets, which were destroyed in diabetic rats (Pillai and Mini, 2016). The study on the antidiabetic potential and insulin-secreting activity of HRS is limited. Therefore, the present study aimed to assess *H. rosa sinensis*’s potential as an antidiabetic agent and look at the consequences associated with diabetes.

**MATERIALS AND METHODS**

**Chemicals**

Streptozotocin (STZ) was procured from Sigma Chemicals.

**Plant extract**

Fresh red-coloured flowers of *H. rosa sinensis* were collected from M. D. University, Rohtak. After that, they were dried in a dark room and then crushed into powder using a grinder. In 300 ml of ethanol, 50 g of this powder was mixed and left for one day. Muslin cloth was used to filter this extract, and the filtrate was then concentrated at room temperature. The reddish waxy residue was obtained and the yield of HRS extract was 15 percent. Then until further analysis, it was stored in a refrigerator (Sankaran and Vadivel, 2011).

**Animals**

Healthy male Wistar albino rats about 3 to 4 months old were purchased from the animal house of Disease Free Small Animal House (DFSAH), Lala Lajpat Rai University of Veterinary & Animal Sciences (LUVAS), Hisar, Haryana. All the laboratory animals were kept under (25 ± 2°C) temperature, 12L: 12D cycle. They were housed in polypropylene cages. They have free access to food and water. The acclimatization period of animals was one week. Eighteen male albino rats were used for this study, divided into three groups (n=6), which the M. D. University, Rohtak Institutional Animal Ethical Committee (IAEC) authorized.

**Induction of diabetes**

Before and after generating diabetes in the animals, their fasting glucose levels were measured. A single 50 mg/kg dosage of streptozotocin in freshly made 0.1 M citrate buffer was used to cause type 1 diabetes in Group 2 and 3 animals (Lina et al., 2017). To overcome hypoglycemia, animals were given a 5% glucose solution after the induction of diabetes. Control (Group 1) animals were only given a single intraperitoneal injection of buffer. After 72 hours of induction, only those animals that exhibited blood glucose levels>250 mg/dl were used for the experiment (Raj et al., 2023). A glucometer was used to measure the blood glucose level.

**Experimental design**

After the successful introduction of Type I diabetes, the animals were divided into following groups.

**Group 1**- Control group, which contained the normal rats.

**Group 2**- Diabetic control group in which animals received intraperitoneal injections of streptozotocin at a dose of 50 mg/kg of body weight (Lina et al., 2017).

**Group 3**- *H. rosa sinensis* treated group in which the diabetic animals were treated with HRS extract at a dose of 125 mg/kg of their body weight daily by oral gavage for 30 days (Sankaran and Vadivel, 2011).

**Histological study**

After one month of treatment, the animals were sacrificed, and the entire pancreas was removed from each group. Organs were preserved in 10% formalin, and pancreatic tissues were processed using paraffin. Afterwards, tissues were sectioned at 5 µm thickness and stained using eosin and hematoxylin. Morphological changes in tissues were examined under a light microscope (Noor et al., 2017).

**Ethical approval**

This study was permitted by the Institutional Animal Ethics Committee (IAEC) at M.D. University, Rohtak.

**RESULTS**

**The success rate of introduction of Type I Diabetes mellitus**

Diabetes was successfully introduced at a dose of 50 mg/kg of STZ, after that these diabetic animals were divided into Diabetic control group (Group 2) and HRS treated group (Group 3). The body weight of animals was significantly reduced after the introduction of Type I diabetes. Glucose levels increased abruptly after the induction of diabetes in comparison to the normal control group (Group 1) (Table 1).

**Changes in body weight and glucose level**

Throughout the experimental period, the body weight of control rats (Group 1) was continuously increasing, but...
the body weight of diabetic rats (Group 2) was significantly decreased (Fig. 1). The *H. rosa sinensis* treated group (Group 3) shows that in the initial half of the experiment, the body weight of animals decreases as compared to the control group (Group 1) animals. However, in the later half of the experiment, the body weight of animals (Group 3) increased as compared to the diabetic control group (Group 2) animals (Fig. 1). On the first day of the experiment, there was no significant difference in the body weight of animals in different groups at p<0.05. But after that, all values are highly significant at p<0.01.

The blood glucose level of the control group (Group 1) animals was < 200 mg/dL throughout the experimental period. The glucose level of the diabetic group (Group 2) animals was > 400 mg/dL (Fig. 2). During the first 10 days of the experiment, the glucose level of HRS treated group (Group 3) animals was comparable to the diabetic group (Group 2) animals. However, after 10 days of treatment, the glucose level of this group of animals (Group 3) was significantly (p<0.01) reduced in comparison to the diabetic control group (Group 2) animals. On the 30th day of the experiment, the glucose level of the HRS-treated group (Group 3) was comparable to the normal control group (Group 1) animals (Fig. 2). All values are significant at p<0.01.

**Histological analysis of pancreas**

The photomicrograph depicts the normal pancreatic structure of control (Group 1) rats (Fig. 3A). The pancreatic acinar cells are well-organized and intact. However, the pancreas of diabetic (Group 2) rats shows severe damage caused by STZ. In this group degeneration and necrosis of pancreatic islets were observed. Due to this, shrinkage of pancreatic acinar cells vacuolation was observed (Fig. 3B). *H. rosa sinensis* treated group (Group 3) shows the improved structure of pancreatic acinar cells. Regeneration of pancreatic islets was observed in this group. The intralubular duct was also well marked like the normal control group (Group 1) (Fig. 3C).

**DISCUSSION**

*H. rosa sinensis* is well known for several properties like antioxidant, antidiabetic, antitumour, antifungal, antibacterial, etc. The present study was planned to estimate the antidiabetic potential of HRS in diabetic

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight</th>
<th>Glucose level</th>
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<tbody>
<tr>
<td></td>
<td>Before induction</td>
<td>After induction</td>
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<tr>
<td>Control group (Group 1)</td>
<td>132.6±3.97</td>
<td>137±2.91*</td>
</tr>
<tr>
<td>Diabetic group (Group 2 &amp; 3)</td>
<td>139±12.22</td>
<td>126.4±11.92*</td>
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All values were represented as Mean ± SD; * Values were significantly different (p<0.05) from control; ** Values were highly significant at p<0.01.
rats. After the induction of diabetes in the rats using streptozotocin, severe weight loss was observed (Table 1) (Kalpana et al., 2021). The reduction in the body weight of diabetic animals (Group 2 & 3) might be because of the deprivation and catabolism of proteins and fats (Patil et al., 2020). The administration of streptozotocin in experimental rats was associated with sudden weight loss (Raj et al., 2022), extreme increase in blood glucose levels (Table 1) and morphological changes in the structure of the pancreas like destruction of insulin-producing beta cells, and degeneration and necrosis of pancreatic islets (Fig. 3 B) (Khin et al., 2023). The diabetic animals treated with HRS extract (Group 3) showed weight gain comparable to that of the normal control (Group 1) rats (Fig. 1). This indicates the affirmative effect of the extract by averting muscle damage. The extract-treated diabetic animals reduced their glucose level to normal at a dosage of 125 mg/kg of body weight (Fig. 2). The mode of action of the extract is still unknown, but the decline in glycemic levels might be due to the regeneration of pancreatic islets (Fig. 3 C) (Ghosh and Dutta, 2017). Venkatesh & Thilagavathi (2008) reported the antidiabetic activity of flower extract of H. rosa sinensis at a dose of 250, 500 mg/kg. But in the present study, the glucose level was reduced in the diabetic animals even at a lower dose of 125 mg/kg. The H. rosa sinensis leaves extract also increases the insulin level in the body in blood plasma.

**Fig. 2.** Changes in the blood glucose level of different groups during the experimental period. All values were represented as Mean ± SD. All values were highly significant at p<0.01.

**Fig. 3.** Represents the histological changes of pancreas of different groups, H&E, 200X. (A) Control group showing the normal intact structure of pancreatic acini (A) and intralobular duct (I). (B) Diabetic control group showing the degeneration and necrosis of pancreatic acinar cells, destruction of pancreatic islets (D) and severe vacuolation (V). (C) Hibiscus rosa-sinensis extract treated group showing the regeneration of pancreatic islets (R), improved structure of pancreatic acinar cells and intralobular duct.
and pancreatic insulin, reducing the glycemic level (Ansari et al., 2020). The ability of HRS leaf extract to secrete insulin was also investigated by Moqbel et al. (2011) and Vimala et al. (2008). Histological study of the pancreas shows the degeneration, vacuolation and necrosis of pancreatic islets in diabetic (Group 2) animals (Fig. 3 B). The destruction of the normal intact structure of pancreatic acini was also observed (Robertson, 2004). The diabetic animals treated with Hibiscus rosa sinensis extract (Group 3) enhanced the structure of pancreatic acinar cells and regeneration of pancreatic islets was also observed (Fig. 3 C). The aforementioned results are consistent with the research conducted by Pillai and Mini (2016), wherein male albino rats (Sprague-Dawley) with diabetes were administered a dose of 250 mg/kg of HRS petal extract. Additionally, Pethe et al. (2017) investigate the improvement in pancreatic islets of alloxan-induced diabetic Wistar albino rats’ size, number, and diameter under treatment with HRS flower extract at doses of 50, 100, and 200 mg/kg. Oxidative stress plays an important role in the pathogenesis of diabetes (Darenskaya et al., 2021). In type I diabetes, reactive oxygen species are involved in the dysfunction of beta cells of the pancreas (Eguchi et al., 2021). HRS flower extract treatment on diabetic animals (Group 3) improves the structure of the pancreas. Pancreatic islets were regenerated in Group 3. The properties of Hibiscus rosa sinensis are likely to contribute to the reported effects. Its antioxidant activity has been known to remove diabetes-induced oxidative stress (Loganathan et al., 2024). In the present study, the antidiabetic activity of flower extract of H. rosa sinensis might be due to the regeneration of pancreatic islets (Fig. 3 C), increasing the insulin secretion from beta cells of the pancreas, thereby reducing the blood glucose level of diabetic animals (Fig. 2).

Conclusion

Hibiscus rosa sinensis can be considered an antidiabetic agent. After inducing diabetes, STZ caused the Wistar albino rats to lose a significant amount of weight. When administered at a dose of 125 mg/kg, HRS flower extract causes diabetic animals to gain weight. Additionally, diabetic animals with HRS flower extract have a glycemic reduction from >400 mg/dL to <200 mg/dL. It was the striking drop in glycemic level after just one month of therapy. Moreover, the extract modifies the deteriorative alterations in the pancreatic tissue of diabetic rats. Pancreatic cell structure was enhanced by HRS flower extract, and pancreatic islet regeneration was also noted. Although more research is required to fully understand the modes of action and long-term effects of H. rosa sinensis flower extract, these results show its promise as an alternative treatment for diabetes.

Conflict of interest
The authors declare that they have no conflict of interest.

REFERENCES
