Hypoglycemic effect of *Hibiscus rosa-sinensis* L. leaf extract in glucose and streptozotocin induced hyperglycemic rats

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Investigations were carried out to evaluate the effect of aqueous extract of *H. rosa-sinensis* leaves on blood glucose level and glucose tolerance using Wistar rats. Repeated administration of the extract (once a day for seven consecutive days), at an oral dose equivalent to 250 mg kg\(^{-1}\), significantly improved glucose tolerance in rats. The peak blood glucose level was obtained at 30 min of glucose load (2 g kg\(^{-1}\)), thereafter a decreasing trend was recorded up to 120 min. The data exhibit that repeated ingestion of the reference drug tolbutamide, a sulphonylurea and the extract brings about 2-3 fold decrease in blood glucose concentration as compared to single oral treatment. The results clearly indicate that tolbutamide improves the glucose tolerance by 91% and extract does so only by 47%. At 250 mg kg\(^{-1}\) the efficacy of the extract was 51.5% of tolbutamide (100 mg kg\(^{-1}\)). In streptozotocin diabetic rats, no significant effect was observed with the extract, while glibenclamide significantly lowered the glucose level up to 7 hr. These data suggest that hypoglycemic activity of *H. rosa-sinensis* leaf extract is comparable to tolbutamide and not to glibenclamide treatment.

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*Hibiscus rosa-sinensis* L. (Malvaceae) is an evergreen woody, glabrous plant well known for its beautiful flowers. It blossoms almost throughout the year and seldom sets seeds under cultivation. The leaves, flowers and roots were found to have medicinal values. Leaves are emollient, refrigerant, anodyne and aperient and have been used\(^1,2\) as an antihyperthermic, antihypertensive and spasmyloytic.

In Kerala, the local women as well as the Ayurvedic physician have been reported to use the leaves for antifertility effect\(^3\). *Hibiscus-mucilage RL*, a representative mucilage isolated for the first time from the leaves of *H. rosa-sinensis*, has been described to show immunological effects on anticomplementary and splenic alkaline phosphatase inducing activity\(^5\). Traditionally the flowers of this plant are attributed with antifertility activity\(^6\) and its benzene extract was found to be most effective. Two major constituents, anthocyanin\(^5\) and \(\beta\)-carotene\(^6\) have been identified from flowers. Raw or pickled flowers are reported\(^7\) to be eaten, in China and Philippines, and a decoction of the root is used for venereal diseases and fevers in Malaya. Significant hypoglycemic activity of *Hibiscus vitifolius* flowers has been indicated in female rats\(^8\). Preliminary investigation of *H. rosa-sinensis* ethanolic leaf extract showed hypoglycemic activity in male albino rats\(^9\).

According to ethnobotanical data *H. rosa-sinensis*, possesses a hypoglycemic effect, which has never been experimentally demonstrated and hence its hypoglycemic potential remains untapped. Therefore, we considered it worthwhile to undertake a study to evaluate the hypoglycemic effect of the aqueous leaf extract of *H. rosa-sinensis* in glucose and streptozotocin induced hyperglycemic rats and compare it with standard hypoglycemic drugs, tolbutamide and glibenclamide, respectively.

Preparation of crude extract—Fresh leaves of *H. rosa-sinensis*, collected in June, were identified and authenticated from Department of Botany, Dayalbagh Educational Institute, Dayalbagh, Agra. After thorough washing, shade dried leaves were powdered and 2.5 g of leaf powder was blended in 100 ml of double distilled water, and left at room temperature with occasional shaking for 16 hr. The extracted material was filtered, and supernatant (crude leaf extract), containing 25 mg ml\(^{-1}\) of the powdered leaves, was ingested at the dose of 250 mg kg\(^{-1}\), to determine its hypoglycemic effect in rats.

Animals—Healthy male albino rats of approximately same age, weighing 150-200 g were used after being acclimatized for a week under laboratory conditions. The animals were maintained on commercial diet (Lipton India Ltd.) and water *ad libitum*.

The hypoglycemic effect was evaluated in 15 normoglycemic rats fasted for 18 hr (allowed free access to water) and randomly divided into three
groups of five animals each. The blood was drawn from orbital plexus of rat and blood glucose was estimated by the glucose oxidase-peroxidase method. Initial glycemia was determined in 18 hour fasted rats. The animals of group 1, receiving vehicle (water) only, served as an untreated control whereas group II and III were given a single oral dose (250 mg kg$^{-1}$) of crude leaf extract and reference drug, tolbutamide (100 mg kg$^{-1}$), respectively. Thirty minutes later a dose of 2 g kg$^{-1}$ of glucose solution was ingested and glucose tolerance test was carried out after drawing blood at 30, 60, 90 and 120 min of glucose load.

Single oral treatment was continued for 7 consecutive days to evaluate the repeated dosing effect. On day 7 glucose tolerance test was performed exactly in the same way as in the single oral treatment.

In 18 hr fasted rats, diabetes was induced by intraperitoneal administration of streptozotocin (STZ, 60 mg kg$^{-1}$) dissolved in 10 mM citrate buffered vehicle (pH 4.5). Antihyperglycemic effect was evaluated, in 18 hr fasted diabetic rats divided into three groups of 5; group I, receiving vehicle only, served as an untreated control, and group II and III were given a single oral dose of the test extract (250 mg kg$^{-1}$) and glibenclamide (100 mg kg$^{-1}$), respectively. Thereafter, blood glucose levels were determined at 0, 1, 3, 5, 7 and 24 hours of treatment and compared in all the groups.

In single oral treatment, at 30 min the blood glucose values increased above control with the extract and decreased under the influence of tolbutamide. At 90 min of glucose load, 22.3 and 9.7% increases in glycemia were observed in control and extract treated groups, respectively. This shows that extract caused 12.6% less increase as compared to controls and attained normal level at 120 min. Tolbutamide was found to bring the blood glucose level to normal after 90 min of glucose load. The glucose tolerance curve after repeated dosing with aqueous extract and tolbutamide, along with normal glucose tolerance curve of rats for comparison, are illustrated in Table 1. The blood glucose level was significantly higher at 30 min of glucose load in control as well as treated groups of animals. Thereafter a decreasing trend in the blood glucose values were recorded upto 120 min. At 90 min, the blood glucose level was 90.5 ± 4.11 mg dl$^{-1}$ which was significantly ($P<0.01$) less than the control level (117.5 ± 4.85 mg dl$^{-1}$) at same time interval. Instead, in tolbutamide treated rats the blood glucose levels were only 110 ± 2.08, 85.75 ± 0.97, 82.25 ± 2.28 and 76.0 ± 1.82 mg dl$^{-1}$ at 30, 60, 90 and 120 min of glucose load, respectively. These values were highly significant ($P<0.01$) at all time intervals as compared to those of control group. These data exhibit that mean percentage rise of blood glucose in the normal control is 43% as against the low percentage rises of 23% and 4% in extract and tolbutamide treated groups, respectively. In STZ rats, a single oral dose of the extract brought about statistically insignificant lowering, while glibenclamide elicited highly significant activity as compared to corresponding vehicle controls (Table 2). Maximum lowering, 22% and 58% were recorded at 1 and 7 hr of the ingestion of extract and glibenclamide, respectively. Extract insignificantly lowered the blood glucose level up to 3 hr, and in contrast glibenclamide showed a

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<tr>
<th>Type of treatment</th>
<th>Blood glucose mg dl$^{-1}$</th>
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<tr>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>Vehicle (Water)</td>
<td>83.50±5.23</td>
</tr>
<tr>
<td>Leaf Extract (250mgkg$^{-1}$)</td>
<td>86.00±5.49</td>
</tr>
<tr>
<td>Tolbutamide (100mgkg$^{-1}$)</td>
<td>84.75±2.17</td>
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</table>

Group 2 and 3 are compared to group 1: $^aP<0.05$, $^bP<0.01$
Group 2 is compared to group 3: $^cP<0.05$, $^dP<0.01$
ANOVA: Comparing the blood glucose values of 3 groups $F(2,60)=57.74$, $P<0.01$
Comparing the values at different time intervals $F(4,60)=83.97$, $P<0.01$
Treatment- time interaction $F(8,60)=9.71$, $P<0.01$

Table 1—Effect of repeated oral administration of H. rosa-sinensis aqueous leaf extract (250mg kg$^{-1}$) and tolbutamide (100mg kg$^{-1}$) on blood glucose concentration in glucose (2g kg$^{-1}$) fed hyperglycemic rats.

Note: Values are mean ± SE of 5 rats. Figures in parentheses are percent glycemia change.
significant lowering trend up to 7 hr. From the results obtained so far it appears that the single oral dose of the leaf extract do not modify the blood glucose levels in STZ diabetic rats, instead repeated oral dosing, of both tolbutamide and the extract in glucose-induced hypoglycemic rats, brings about 2-3 fold decrease in the blood glucose level as compared to single oral treatment. These values suggest that if the drug improves the glucose tolerance by 91% the extract does so by 47%. Such an improvement in the ability of animals to utilize an external glucose load under the influence of plant extract seems to suggest that *H. rosa-sinensis* may have a mechanism of action similar to that of sulphonylurea rather than to that of biguanides.

Further comprehensive chemical and dose-time dependent investigations are needed to elucidate the exact mechanism of hypoglycemic effect of *H. rosa-sinensis*.

**References**


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**Table 2**—Effect of single oral administration of *H. rosa-sinensis* aqueous leaf extract (250 mg kg$^{-1}$) and glibenclamide (10 mg kg$^{-1}$) on blood glucose concentration in streptozotocin induced hyperglycemic rats

<table>
<thead>
<tr>
<th>Type of treatment</th>
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<tr>
<td></td>
<td>0 hr</td>
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<tr>
<td>Vehicle</td>
<td>291.40</td>
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<tr>
<td>(Water)</td>
<td>± 5.08</td>
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<tr>
<td>Leaf extract</td>
<td>265.20</td>
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<tr>
<td>(250 mg kg$^{-1}$)</td>
<td>± 15.49b</td>
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<tr>
<td>Glibenclamide</td>
<td>409.00</td>
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<tr>
<td>(10 mg kg$^{-1}$)</td>
<td>± 20.02ab</td>
</tr>
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Group 2 and 3 are compared to group 1: $^aP<0.05$, $^bP<0.01$

ANOVA: Comparing the blood glucose values of 3 groups F (2,72)=3.57, NS

Comparing the values at different time intervals F (5,72)=4.33, NS

Treatment-time interaction F (10,72)=7.10, P<0.01