

## Original Research Article

# Anti-hyperglycemic activity of *Heliotropium strigosum* (Boraginaeae) whole plant extract in alloxan-induced diabetic mice

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### Abstract

**Purpose:** To investigate *Heliotropium strigosum* whole plant extract for its potential to reduce the blood glucose level of alloxan-induced diabetic mice.

**Methods:** Preliminary phytochemical analysis was carried out using standard procedures. Diabetes was induced in Balb/c mice by injecting alloxan (200 mg/kg i.p.). The crude methanol extract of *Heliotropium strigosum* (Hs.Cr, 30, 100 and 300 mg/kg doses) was administered daily to alloxan-induced diabetic mice for 15 days and its effect on fasting blood glucose levels, body weight and oral glucose tolerance was evaluated. Two control groups (non-diabetic control and diabetic control) received normal saline (0.2 ml). Metformin (500 mg/kg) was used as reference standard.

**Results:** *Heliotropium strigosum* showed positive for the presence of alkaloids, tannins and flavonoids. The extract (30, 100 and 300 mg/kg) caused significant reduction in the fasting blood glucose level of alloxan-induced diabetic mice on days 5, 10 and 15 compared to diabetic control ( $p < 0.001$ ). In this regard, the anti-hyperglycemic effect compared to the reference (metformin). The extract also time-dependently decreased the body weight of the treated animals as well as improved tolerance of the oral glucose overload.

**Conclusion:** These results indicate that *Heliotropium strigosum* possesses anti-hyperglycemic effect, reduces body weight and enhances the tolerance of glucose overload in mice. Further studies are therefore required to determine its feasibility as an alternate herbal medicine in the management of diabetes in humans.

**Keywords:** *Heliotropium strigosum*, Anti-hyperglycemic, Alloxan-induced diabetic mice, Blood glucose, Oral glucose tolerance, Body weight

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## INTRODUCTION

Diabetes mellitus is a metabolic disorder that tends to cause hyperglycemia due to inefficient working of the beta cells or resistance towards the insulin at the target tissues [1]. Diabetes causes microvascular and macrovascular

complications. These include retinopathy, nephropathy, cardiovascular and cerebrovascular diseases. Until 2013 382 million people had been suffering from diabetes and it is expected that the numbers may increase to 592 million by 2035 [2]. Oral anti-diabetics with variant mechanisms along with injectable insulin and

insulin preparations are currently in use [3]. World Health Organization has permitted the use of plants with medicinal values as an alternate therapy [4]. According to estimation by Alarcon *et al* [5], only 350 species of plants have been scientifically validated out of 800 species with expected anti-diabetic potential.

*Heliotropium strigosum* (known as 'bhangra' and 'kharsan' in Pakistan) belongs to the family Boraginaeae. It has been used in the traditional system of medicine for the treatment of GI ailments, bites, boils, eyesores, ulcers and imbalance hormonal functions [6]. Anti-microbial, anti-oxidant [7], anti-inflammatory, analgesic, anticonvulsant [8], cytotoxic, phototoxic [9], spasmolytic, bronchodilator and vaso-relaxant actions of *Heliotropium strigosum* have been reported [10]. It contains strigosine as major alkaloid [11] and a trypsin inhibitor, *phthalic acid* ester, has also been isolated. The aerial parts of *Heliotropium strigosum* obtained from Punjab Province has been investigated for anti-diabetic activity on rats [12].

In present study, the objective was to investigate the whole plant as an antihyperglycemic agent in Balb/c mice using different models and doses.

## EXPERIMENTAL

### Plant material and extraction

The whole plant of *Heliotropium strigosum* was collected from Malakand Agency, Pakistan. Dr. Zafar Iqbal of Kohat University of Science and Technology, KPK, Pakistan authenticated it, and a voucher specimen (no. 1230-B) was deposited in herbarium at the same department. The plant material was kept in shade at room temperature for drying. The coarse powder was obtained by grinding the shade-dried whole plant at room temperature. Obtained powder was kept for maceration in 70 % methanol for 7 days with regular shaking and mixing at intervals [10]. The resultant methanol extract was concentrated on rotary evaporator under reduced pressure to obtain *Heliotropium strigosum* crude extract (Hs.Cr).

### Chemicals

All chemicals used in this study were of highest analytical grade. Metformin hydrochloride was obtained as a gift from Caraway Pharmaceuticals, Rawat, Islamabad. However, Alloxan monohydrate (inducing agent) and methanol (maceration) were purchased from *Sigma-Aldrich Co.* LLC and respectively.

### Animals

In this study Balb-C mice (25 - 30 g) were used. Mice were housed at the Animal House of Riphah Institute of Pharmaceutical Sciences, where room temperature was maintained at 23 - 25 ° C . Mice were fed with standard diet and tap water. The rules for experimental use of animals complied with guidelines provided by "Principles of Laboratory Animal care" (NIH publication 85-23, revised 1985) [13]. Ethical approval was obtained prior to experimentation from Ethical Committee of Riphah Institute of Pharmaceutical Sciences, Riphah International University (ref no. REC/RIPS/2015/002).

### Preliminary phytochemical screening

Hs.Cr was analysed for presence of phytoconstituents like alkaloids, tannins, proteins, steroids and flavonoids through preliminary phytochemical analysis according to standard procedures [14]. Alkaloids were detected by using Dragendorff's and Mayer's reagent. Flavonoids tested using sodium hydroxide test. Tannins were detected using lead acetate test. Ninhydrin was used for protein detection. Steroids were tested using chloroform test.

### Assessment of blood glucose level and body weight

Mice were acclimatized to lab conditions. Mice were pre-fasted for 12 h and injected with freshly prepared solution of alloxan (200 mg/kg) intraperitoneal for induction of diabetes [15]. After 48 h of alloxan induction mice with fasting blood glucose levels above 200 mg/dL along with signs of polyuria and polydipsia were considered diabetic and included in the study. Selected mice were divided into 5 groups (n=5). Group I (non-diabetic mice normal control given saline), Group II (diabetic control mice) diabetic mice served saline, Group III, IV and V include diabetic mice that received 30, 100 and 300 mg/kg doses of Hs.Cr respectively. Group VI comprised of diabetic mice receiving standard drug metformin (500 mg/kg). All groups were treated respectively for 15 consecutive days. EASYGLUCO Auto-coding Glucometer and weighing balance were used for monitoring blood glucose levels and body weight (g) at regular intervals.

### Oral glucose tolerance test

The selected mice were kept on fasting for 14-15 h and divided in non-diabetic control group, diabetic control group, test and standard drug treatment groups. The diabetic and non-diabetic control groups were administered saline, test

group was given Hs.Cr (300 mg/kg) and standard group received metformin (500 mg/kg) 30 min prior to administration of oral D-glucose (2 g/Kg). Blood was withdrawn at 0 min (before glucose load), then at 30, 60, 90 and 120 min after glucose administration for assessment of blood glucose level using tail-prick method [16].

### Acute toxicity test

The oral toxicity test with acute doses was performed [17]. The overnight fasting mice (n = 6) in each group were treated with Hs.Cr orally at acute doses of 1, 3 and 5 g/ Kg. Mice were observed for 48 h and any signs of Gi discomfort, diarrheal spots, discomfort, neurological/behavioral changes, convulsions, coma or death were recorded.

### Statistical analysis

The results are expressed as mean  $\pm$  standard error of mean (SEM, n = 5). One-way analysis of variance (ANOVA) followed by Tukey post-hoc test is used as statistical parameter.  $P < 0.05$  was set as significantly different, and GraphPad program (GraphPad, San Diego, CA, USA) was used for analyses of data.

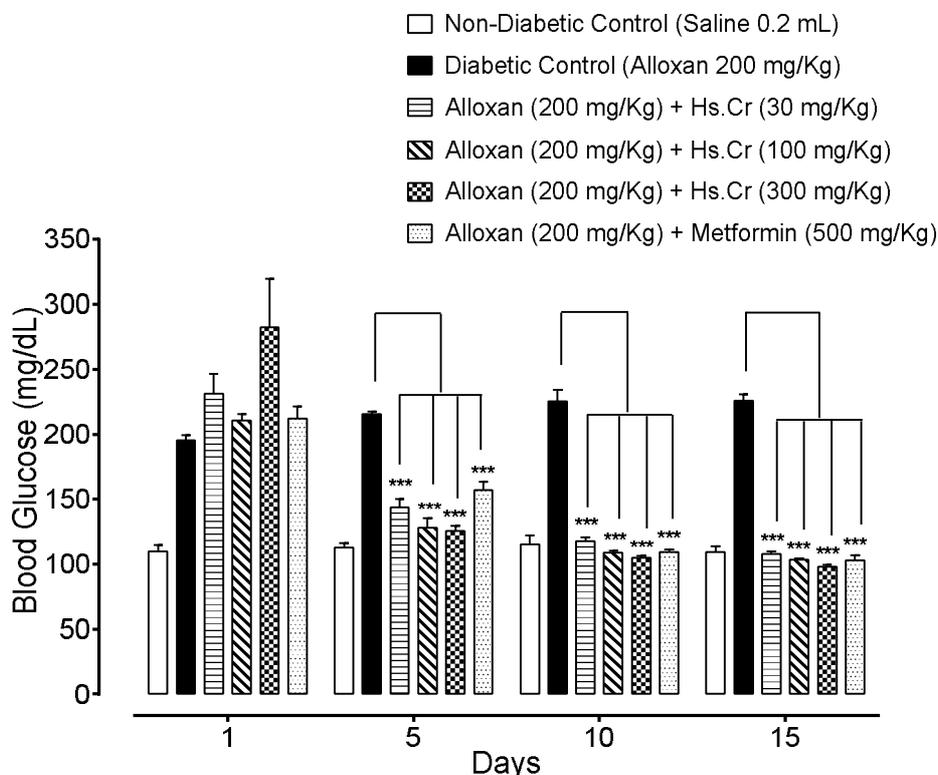
## RESULTS

### Phytochemical analysis

Hs.Cr tested positive for the presence of alkaloids, tannins and flavonoids.

### Effect of Hs.Cr on blood glucose level

Hs.Cr at doses of 30, 100 and 300 mg/kg significantly reduced the blood glucose of alloxan-induced diabetic mice (Figure 1), similar to what was seen in the metformin group. The animals of non-diabetic normal control group had fasting blood glucose levels of  $109.4 \pm 4.1$  mg/dl at day 15 whereas that of diabetic control group was  $225.6 \pm 5.6$  mg/dl. The group treated with Hs.Cr (30 mg/kg) showed significant reduction of blood glucose levels of  $107.6 \pm 2.4$  mg/dl ( $***p < 0.001$  vs. diabetic control group) at day 15. At day 15 the dose of of Hs.Cr (100 mg/kg) significantly reduced blood glucose levels to  $103.2 \pm 1.4$  mg/dl ( $***p < 0.001$  vs. diabetic control group). The blood glucose levels of Hs.Cr (300 mg/Kg) showed a remarkable significant reduction to  $98 \pm 1.7$  mg/dl ( $***p < 0.001$  vs. diabetic control group) which is even better than metformin (500 mg/Kg) treated group that reduced blood glucose levels to  $103 \pm 3.9$  mg/dl ( $***p < 0.001$  vs. diabetic control group) at day 15.

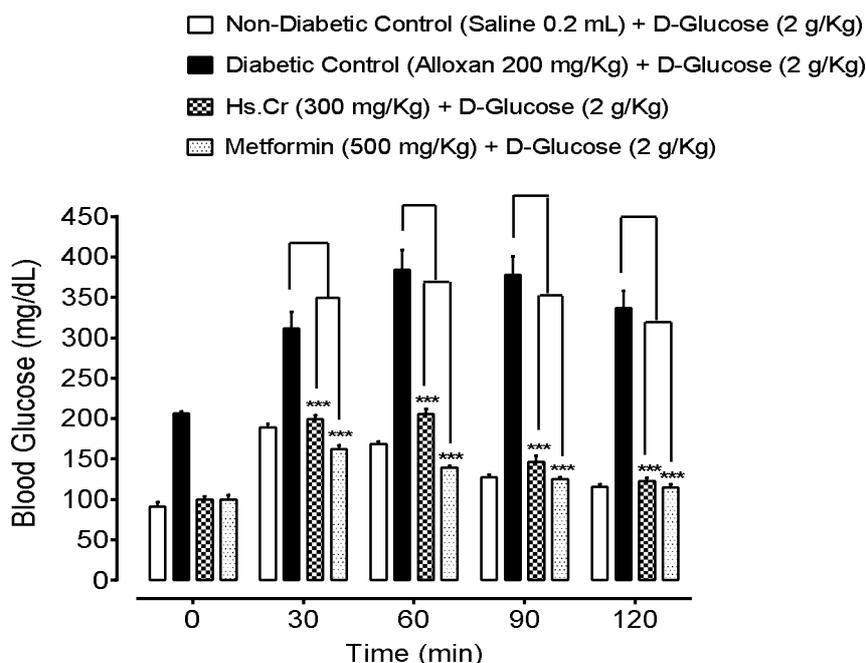


**Figure 1:** Bar-graph representing the blood glucose levels at different treatment days. Data is represented as mean  $\pm$  SEM (n = 5);  $***p < 0.001$  was obtained by comparison of the blood glucose values of treated groups to that of diabetic control group, one-way ANOVA followed by Tukey's test

**Table 1:** Effect of *Heliotropium strigosum* crude extract (Hs.Cr) and metformin on the body weight of alloxan-treated diabetic mice

Treatment	Day 1	Day 5	Day 10	Day 15
Non-diabetic control (saline 0.2 ml)	32.60 ± 0.6	32.46 ± 0.7	31.70 ± 0.8	32.21 ± 1.5
Diabetic control (200 mg/kg) + (saline 0.2ml)	28.92 ± 1.6	28.22 ± 1.7	27.44 ± 1.6	27.40 ± 1.6
Alloxan (200 mg/kg) + Hs.Cr (30 mg/kg)	21.2 ± 0.6	20.8 ± 0.7	19.9 ± 0.3	19.5 ± 0.4
Alloxan (200 mg/kg) + Hs.Cr (100 mg/kg)	36.7 ± 1.7	35.6 ± 1.9	33.8 ± 1.7	33.1 ± 1.7
Alloxan (200 mg/kg) + Hs.Cr (300 mg/kg)	35.3 ± 1.4	33.6 ± 1.7	30.3 ± 1.1	29.9 ± 1.1
Alloxan (200 mg/kg) + Metformin (500 mg/kg)	24.6 ± 1.8	24.2 ± 1.7	23.7 ± 1.8	23.2 ± 1.8

Values are mean ± SEM (n = 5)



**Figure 2:** Bar-graph representing the blood glucose levels before and after administration of oral glucose load in mice. Data represented as mean ± SEM (n = 5); \*\*\* $p < 0.001$  is obtained with Hs.Cr and metformin treated groups vs. alloxan-induced diabetic control group. one-way ANOVA followed by Tukey's test

### Effect of Hs.Cr on body weight

Hs.Cr at doses of 30, 100 and 300 mg/kg significantly reduced body weight of treated animals at days 5, 10 and 15<sup>th</sup>, compared to the initial body weights of each group. These effects of weight reduction were similar to that of metformin group (Table 1).

### Effect of Hs.Cr on glucose tolerance

Hs.Cr at dose of 300 mg/kg significantly improved the tolerance of orally administered glucose ( $^{***}p < 0.001$  vs. diabetic control group), similar to that caused by metformin. After glucose (2 g/kg) overload, blood glucose levels of non-diabetic, diabetic, Hs.Cr (300 mg/kg) and metformin (500 mg/kg) treated group were recorded to be 115.6 ± 3.3 mg/dL, 336.8 ± 21.5 mg/dL, 122.8 ± 3.7 mg/dL ( $^{***}p < 0.001$  vs. diabetic control group) and 114.6 ± 3.9 mg/dL ( $^{***}p < 0.001$  vs. diabetic control group) respectively at 120 mins (Figure 2).

### Acute toxicity

Hs.Cr was safe even at doses as high as 3 g/kg. However, death was observed only at the dose of 5 g/kg orally.

## DISCUSSION

In the present study, anti-diabetic potential of *Heliotropium strigosum* against alloxan-induced diabetes in mice was investigated. Alloxan is used as a toxic agent for induction of diabetes. It causes diabetes by destruction of pancreatic beta cells through necrosis due to oxidative stress induced by formation of reactive oxygen species. This leads to inhibition of insulin secretion resulting in persistent hyperglycemia or diabetes [18]. *Heliotropium strigosum* caused significant decrease in blood glucose levels of mice in dose-dependent and time-dependent fashion when compared to alloxan-treated diabetic group and standard group treated with metformin. Metformin is a biguanide oral anti-

diabetic drug in market which is used as a standard drug [19].

Metformin lowers the glucose levels by acting through numerous paths. This includes reducing hepatic glucose production, limiting glucose absorption in intestine and improving glucose uptake and utilization through enhancing insulin sensitivity [20]. In same pattern of metformin the plant extract also caused reduction in body weights of test mice measured at regular day intervals. Diabetes is often associated with obesity and it is known to be one of the major risk factors for diabetes, hence a drug with dual benefits of glycemic and weight control is of interest in diabetes associated with obesity. Hence it is a positive outcome of *Heliotropium strigosum* usage. Metformin also possess both weight reduction and anti-hyperlipidemic properties and hence is a drug of choice in diabetes associated with obesity [21]. In glucose-loaded hyperglycemia model, which tends to assess oral glucose tolerance, Hs.Cr displayed considerably better tolerance of glucose overload in experimental intervals as does the metformin group. Excess of glucose in blood impels insulin secretion. The resultant insulin stimulates glucose uptake and use peripherally and controls processing of glucose through numerous mechanisms [17]. Hs.Cr produced lowering of blood glucose, body weight and enhanced glucose overload tolerance in a similar pattern to that of metformin, suggesting that *Heliotropium strigosum* may also have possibly mediated anti-diabetic effect through aforementioned mechanism(s) of actions exerted by metformin. Qayyum et al [22] reported amylase inhibitory potential of *Heliotropium strigosum*. However, further advance molecular studies need to be conducted for elucidation of underlying pharmacodynamics involved. The anti-diabetic effect of *Heliotropium strigosum* observed may be due to the effect of rich phyto-constituents as alkaloids and flavonoids that possess hypoglycemic potential and are considered useful for treating diabetes as well as to reduce complication associated with it [23]. Major diseases occur due to the excessive oxidative stress induced by cellular injury [24]. Hs.Cr has been validated to possess antioxidant activity that makes it beneficial in reducing oxidative stress, which may be one of the prospected reasons of its effectiveness as an antidiabetic.

## CONCLUSION

*Heliotropium strigosum* shows anti-hyperglycemic effect in alloxan-induced diabetic mice and improves acute glucose tolerance. Thus, these findings study provide scientific

justification of *Heliotropium strigosum* in the management of diabetes mellitus in traditional medicine system of Pakistan. Further studies will be carried out in future to identify the active anti-diabetic principles of this plant that are responsible.

## DECLARATIONS

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### Conflict of Interest

No conflict of interest associated with this work.

### Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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