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Research Article

# *In vitro* α-Amylase Inhibition and Antihyperglycemic Activity of *Helicteres isora* in Streptozotocin-Induced Rats

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**ABSTRACT**
In present study, *in vitro* α-amylase inhibition and streptozotocin-induced oxidative stress were used to evaluate hypoglycemic effects of *Helicteres isora* (sterculiaceae). Antidiabetic treatment with of *H. isora* extract (100 mg/kg and 250 mg/kg body weight) for three weeks showed significant reduction in thiobarbituriuc acid reactive substances (TBRAS) and glutathione reductase (GSH-R) in both liver and kidney. The treatment with *H. isora* significantly altered the glutathione (GSH) and GSH-R to be comparable with the control group. *H. isora* and tolbutamide treated rats showed decreased lipid peroxidation that is associated with increased activity of superoxide dismutase (SOD) and catalase (CAT).The result of this study thus shows that at 1000 µg /ml there was maximum inhibition by the *H. isora* extract (53.75 %). It also possesses moderate antidiabetic activity, but it exhibits potent antioxidant potential in diabetic conditions.

**Keywords:** α-amylase inhibition, antidiabetic, streptozotocin, antioxidant, oxidative stress

## INTRODUCTION

It has been assumed that the etiology of the complication of diabetes involves oxidative stress perhaps as a result of hyperglycemia. [1] The elevated levels of blood glucose in diabetes produce oxygen free radicals which cause membrane damage due to peroxidation of membrane lipids and protein glycation. [2] Numerous studies have been demonstrated that oxidative stress, mediated mainly by hyperglycaemia-induced generation of free radicals, contributes to the development and progression of diabetes and its complications. [3] Abnormally high levels of free radicals which cause membrane damage due to peroxidation of membrane lipids and protein glycation and the simultaneous decline of antioxidant defense mechanisms leads to cell and tissue damage. [4]

*H. isora* (Sterculiaceae) is a woody shrub or small tree 2-3 m tall, young shoots tellate hairy, bark thin and strong. In India it is distributed from Jhelum Eastwards to Nepal, Bihar, West Bengal, Central, Western and Southern India.The roots and the bark are expectorant, demulcent and are useful in colic, scabies, gastropathy, diabetes, diarrhea and dysentery. [5] The wood which is twice as hard as teak, is extensively used for making boats, carts, carriages, firewood, bows of violin, planking, tool-handles, beams and fence posts. [6] From the roots; betulic acid, daucosterol, sitosterol, isorin were isolated by Qu and Wang (1991). Earlier chemical examinations on *H. isora* roots have shown the presence of Cucurbitacin B and isocucurbitacin B were isolated and reported to possess cytotoxic activity. [7-8] Isolation and characterization of bioactive terpene from the fruits of *H. isora* were studied by Sandhya and Grampurohit, 2007. [9] The aqueous extract of bark of *H. isora* plant is reported to have antioxidant activity. [10-11] The $LD_{50}$ value of *H. isora* bark is found to be greater than 2000 mg/kg in rats. [12] Bark extracts of H. *isora* posses to hypolipidaemic activity in streptozotocin induced diabetic rats. [13] However, to till date no α-amylase inhibition *in vitro* and antioxidant investigations in experimental animals have been reported in this plant. Therefore, the present research work was undertaken for the study

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## MATERIALS AND METHODS

### Plant materials

Roots of H. *isora* were collected from Srisailam forest, Andhara Pradesh, India. The plant material were identified taxonomically and authenticated by National Botanical Research Institute, Lucknow. A voucher specimen of the collected sample was deposited in the institutional herbarium for future reference.

### Preparation of 50 % ethanolic extract

The shade dried plant materials (Root) was crushed, powdered and exhaustively extracted by overnight maceration with 10 volumes of 50 % ethanol. The extracts were filtered, pooled and concentrated on rotavapour (Buchi, USA) and dried in lyophilizer (Laboconco, USA) under reduced pressure to obtain 10 % of solid residue.

### Preliminary phytochemical analysis

From preliminary phytochemical analysis it was found that *H. isora* showed positive tests for major primary and secondary plant metabolites except alkaloid, carbohydrate, protein and starch.

***In vitro* α-amylase inhibition** Assay method was adopted and modified from Sigma-Aldrich. [14] A starch solution (0.5 % w/v) was obtained by stirring 0.125 g of potato starch in 25 ml of 20 mM sodium phosphate buffer with 6.7 mM sodium chloride, pH 6.9 at 65°C for 15 minutes. 2 unit/ml enzyme

solution was prepared by mixing α-amylase in ice-cold distilled water. Plant extract are dissolved in buffer to final concentration from 1 mg/ml to 100 µg/ml. Colour reagent was prepared by mixing a sodium potassium tartrate solution (12.0 g of sodium potassium tartrate, tetrahydrate in 8.0 ml of 2M NaOH) and 96 mM 3,5-dinitrosalicylic acid solution. Both control and plant extracts were added with starch solution and left to react with α-amyalse solution for 3 minutes. Reaction mixture was mixed with dinitrosalicylic acid solution and kept in water bath at 85°C for 15 minutes. One unit of α-amylase liberates 1.0 mg of maltose from starch. The generation of maltose was quantified by the reduction of 3, 5-dinitrosalicylic acid to 3-amino-5-nitrosalicylic acid (colour change from orange to yellow) and quantified at 540 nm. Plant extract without the amylase was also used as the control and the corrected value of the sample absorbance noted.

Standard calibration curve for maltose was plotted against concentration of maltose (10- 100 µg) and absorbance of the solution at 540 nm. The α-amylase inhibition was expressed as percentage of inhibition and calculated by the following equations:

**Percentage Reaction**= [Maltose in test / Maltose in control] × 100

**Percentage Inhibition**= 100- Percentage Reaction

**Animals**: Sprague-Dawley rats (150-175 g) and albino mice (20 - 25 g) were obtained from the animal colony of National Laboratory Animal Centre, Lucknow. They randomly distributed into various groups and housed in cages (6 per cage) and maintained under standard conditions i.e. 26 ± 2°C and relative humidity 44-56 % and 10 h light: 14 h dark cycles each day for one week before and during the experiments. All animals were fed standard rodent pellet diet (Amrut, India) and drinking water *ad libitum*. All studies were performed in accordance with the guide for the care and use of laboratory animals, as adopted and promulgated by the Institutional Animal Care Committee, CPCSEA, India (Reg. No. 222/2000/CPCSEA).

**Experimental procedure**

*Induction of diabetes*- Diabetes was induced in experimental rats by injecting streptozotocin (50 mg/ kg, body weight, i.p). Diabetes was confirmed in STZ rats by measuring the fasting blood glucose concentration after 96 h after the injection of STZ. The rats with blood glucose level above 140mg/dl were considered to be diabetic and were used in the experiment. The animals are kept fasting over night and the extract was injected to study the anti-diabetic activity. [15]

Experiment design- After induction of diabetes, the rats was divided into 5 groups.

Group I-Control rats received vehicle solution

Group II-Diabetic control

Group III-Diabetic rats treated with *H. isora* extract 100mg/kg b.w. in 2% gum acacia

Group IV- Diabetic rats treated with *H. isora* extract 250mg/kg b.w. in 2% gum acacia

Group V- Diabetic rats treated with tolbutamide in 10mg/kg, sigma USA.

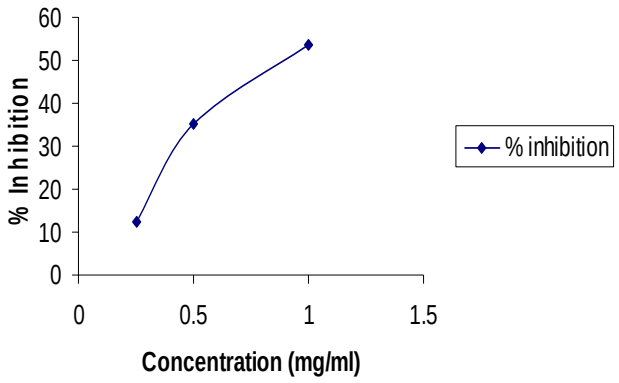
*Estimation of blood glucose:* The vehicle and the drugs were administered orally using intra gastric tube daily for three weeks. After three weeks of treatment the rats were fasted overnight, the blood samples were analyzed for blood glucose content was determined by the o-toludine method. [16] With optical density measured by visible spectrophotometer at 520 nm. Then the animal was sacrificed by cervical decapitation. The liver and kidney was exposed and perfused with cold phosphate buffer saline of pH 7.4. Blood free liver was taken out and homogenized in a glass Teflon homogenizer (10 %w/v).Incubation were done at 37°C under controlled conditions.

**Antioxidant activities:** Lipid peroxidation in liver and kidney were estimated colorimetrically by thiobarbituric acid reactive substances (TBRAS) [17] and hydroperoxides by the method of Jamall and smith (1985). [18] Glutathione (GSH) was estimated using Butler et al. (1967) [19], Glutathione reductase (GSH-R) was estimated using standard method of Horn (1963). [20] Superoxide dismutase (SOD) was measured by using the methods of Kakkar *et al.* (1984). [21] Catalse (CAT) activity was measured by using the rate of decomposition of $H_2O_2$ by the method of Aebi (1974). [22] All these estimations were made in both liver and kidney.

**Statistical analysis**

All the data were presented as mean ± S.D and data were analyzed by paired-*t*-test using SPSS software package. (SPSS, Cary, NC, USA)

**Fig. 1: Inhibitory activity of extract against α-amylase**




**RESULTS**

The different doses of the extract were studied to study the dose response. At 1000 µg /ml there was maximum inhibition by the extract, with *H. isora* (53.75 %) and data are represented in figure 1.

There was a moderate decrease in the blood glucose level of diabetic rats upon administration of *H. isora* (Table 1). Table 2 shows the levels of TBARS, GSH and GSH-R in liver and kidney of control and experimental animals. A significant elevation in tissues TBARS, GSH, and GSH-R was observed in the diabetic control rats as compared to the normal control rats. Oral administration of *H. isora* extract (100 and 250 mg/kg body weight) for three weeks shows significant reduction in TBARS and GSH-R in both liver and kidney. With respect to GSH there was a significant reduction in the glutathione in the liver while no significant reduction was observed in the kidney. The results were comparable to that of standard drug.

Table 2 shows the activities of the enzymatic antioxidants SOD and CAT in liver and kidney. Activities of these enzymes decreased significantly in the diabetic control rats as compared to the normal control. Oral administration of *H. isora* extract (100 mg and 250 mg/kg body weight) for three weeks significantly reversed these enzymes to near normal values.

**Table 1: Effect of *H. isora* extract on serum glucose level in STZ diabetic rats**

| Groups | Treatment P.O. | Blood glucose (mg/ dl) | | |
|---|---|---|---|---|
| | | 0 day | 7th day | 21st day |
| I | Normal | 78.21±11.41 | 77.46± 10.32 | 78.86 ± 9.34 |
| II | Control (Diabetic) | 247.22±33.56 | 229.46±12.11$^{c}$ | 210.32 ± 21.12$^{c}$ |
| III | Aq. EtOH extract (100mg/kg) | 239.36± 10.64 | 167.46±13.26$^{y}$ | 121.28 ± 13.14$^{z}$ |
| IV | Aq. EtOH extract (250mg/kg) | 255.13± 11.25 | 121.32±12.40$^{z}$ | 103.24 ± 11.22$^{z}$ |
| V | Tolbutamide (10mg/kg) | 257.32± 12.21 | 105.12±12.60$^{z}$ | 91.18 ± 12.28$^{z}$ |

The values represent the means ± S. E. M for six rats per group. $^{x}p<0.05$, $^{y}p<0.01$ and $^{z}p<0.001$ compared to diabetic control group. $^{c}p<0.001$ as compared to normal

**Table 2: Effect of *H. isora* extract on antioxidant enzyme activities in STZ diabetic rats**

| Parameters | Normal control | Control (Diabetic) | HIEt (100mg/kg) | HIEt (250mg/kg) | Tolbutamide (10mg/kg) |
|---|---|---|---|---|---|
| **TBARS (Nmoles of MDA/mg protein)** | | | | | |
| Liver | 5.71 ± 0.4 | 6.53 ± 0.13$^{c}$ | 6.09 ± 0.05$^{x}$ | 6.20 ± 0.06$^{y}$ | 6.91 ± 0.10$^{x}$ |
| Kidney | 7.21 ± 0.06 | 8.78 ± 0.21$^{c}$ | 8.30 ± 0.12$^{x}$ | 8.01 ± 0.10$^{y}$ | 7.99 ± 0.13$^{y}$ |
| **Glutathione (nM of DTNB conjugated/mg protein)** | | | | | |
| Liver | 131.13 ± 2.27 | 75.25 ± 3.52$^{c}$ | 85.42 ± 3.85$^{x}$ | 105.44 ± 2.35$^{z}$ | 123.78 ± 2.44$^{z}$ |
| Kidney | 123.55 ± 3.19 | 65.32 ± 2.56 | 75.13 ± 3.57$^{x}$ | 82.84 ± 2.53$^{y}$ | 88.18 ± 3.23$^{z}$ |
| **Gluthione Peroxidase (U/mg protein)** | | | | | |
| Liver | 208.97 ± 0.53 | 206.30 ± 0.47$^{b}$ | 208.49 ± 0.53$^{y}$ | 209.71 ± 0.66$^{z}$ | 209.11 ± 0.22$^{y}$ |
| Kidney | 217.52 ± 0.44 | 215.12 ± 1.23$^{b}$ | 216.13 ± 0.41$^{x}$ | 217.30 ± 0.57$^{x}$ | 215.58 ± 0.23$^{x}$ |
| **CAT (unit/mg protein)** | | | | | |
| Liver | 70.26 ± 2.3 | 37.64 ± 1.81$^{c}$ | 49.43 ± 3.57$^{y}$ | 74.21 ± 1.25$^{z}$ | 66.30 ± 2.52$^{z}$ |
| Kidney | 39.32 ± 1.25 | 20.57 ± 1.77$^{b}$ | 25.64 ± 1.21$^{x}$ | 35.55 ± 1.43$^{z}$ | 34.27 ± 1.83$^{z}$ |
| **SOD (unit/mg protein)** | | | | | |
| Liver | 6.34 ± 0.19 | 11.41 ± 0.15$^{c}$ | 13.90 ± 0.62$^{x}$ | 15.40 ± 1.43$^{y}$ | 14.818 ± 0.31$^{y}$ |
| Kidney | 7.7±0.41 | 12.7±0.48$^{c}$ | 11.3±0.12$^{x}$ | 13.6±0.10$^{y}$ | 14.3±0.08$^{y}$ |

The values represent the means ± S. E. M for six rats per group. $^{x}p<0.05$, $^{y}p<0.01$ and $^{z}p<0.001$ compared to diabetic control group. $^{b}p<0.01$, $^{c}p<0.001$ as compared to normal; SOD: superoxide dismutase; GSH: Glutathione; GPx: glutathione peroxidase; GST: glutathione-S-transferase;CAT:Catalase

## DISCUSSION

The digestion of carbohydrates a fundamental process for the provision of energy requires hydrolysis of the glycosidic bonds in polysaccharides e.g. starch. The enzymes responsible for catalysis are denoted as "amylolytic". α-Amylases are considered as endo-acting enzymes which randomly attack internal α-1,4-glycosidic linkages, except for those adjacent to the ends of the substrate and those near the branch points, the products of this reaction are maltose, maltotriose and α-dextrins. Comparatively, α-amylases are classed as exo-acting hydrolysing α-1, 4 bonds from the non-reducing ends of the substrate thus producing α-products. In monosaccharide glucose can be readily absorbed from the GI tract into the blood stream after the hydrolysis of glycosidic bonds in digestible carbohydrate foods containing starch by the enzyme α-amylase. The inhibition of these enzymes reduces the high post parandial blood glucose in diabetics. [23]

Lipid Peroxidation is a free radical induced Process leading to oxidative deterioration of polyunsaturated fatty acids. Under Physiologic condition, low concentrations of lipid peroxides are found in tissues. Karpen *et* al ., (1982) [24] observed an elevated level of lipid peroxides in the plasma of diabetic rats and lipid peroxidation as one of the characteristic features of chronic diabetes. Increased levels of lipid Peroxides has also been reported in the kidney of diabetic rats and increased levels of TBARS as an index of lipid peroxidation. [25]

The involvement of free radicals in diabetes and the role of these toxic species in LPO and the antioxidant defense system have been studied. Depletion of tissue glutathione and increase in lipid peroxidation has been observed in diabetes. [26] It has been proposed that antioxidants that maintain the concentration of reduced glutathione may restore the cellular defense mechanisms, block lipid peroxidation and thus protect the tissue damage against oxidative damage. [27] Our results show that in diabetic control animals the level of TBARS was high due to increased lipid peroxidation. In *H. isora* and tolbutamide treated diabetic rats ,the TBARS levels decreased both in liver and kidney which may be due to the free radical scavenging action of the active ingredients present the *H. isora* extract inhibited the radiation induced lipid peroxidation process effectively and that could be attributed to the ability to scavenge the free radicals involved in the initiation and propagation steps.

Reduction of oxidized from of glutathione requires NADPH, a cofactor and enzyme glutathione reductase. The reduced availability of NADPH which could be either due to reduced synthesis or increased metabolism of NADPH through some other pathway, could be responsible for low levels of reduced glutathione in streptozotocin treated rats as compared to control rats. One of the consequences of hyperglycemia is increased metabolism of glucose by sorbitol pathway. Besides this, other pathway such as fatty acid and cholesterol biosynthesis also compete for NADPH with GSH. The significant increase in the GSH content and GSH-R activates in tissue in diabetic rats indicates an adaptive mechanism in response to oxidative stress. [28] The treatment with *H. isora* significantly altered the GSH and GSH-R to be comparable with the control group.

SOD scavenges the superoxide ions produced as cellular byproducts. SOD is a major defense for aerobic cells in combating the toxic effects of superoxide radicals. [29] CAT reduces Hydrogen per oxide produced by disputation reaction and preventing generation of hydroxyl radicals thereby

protecting the cellular constituents from oxidative damage in peroxisomes. SOD and CAT are two scavenging enzymes that remove the toxic free radical in vivo. Reduced activities of SOD and CAT in liver and kidney have been observed during diabetes and this may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide. [30] *H. isora* and tolbutamide treated rats showed decreased lipid peroxidation that is associated with increased activity of SOD and CAT. The result of this study thus shows potent α-amylase inhibition by the *H. isora*. It possesses moderate antidiabetic activity whereas exhibits potent antioxidant potential in streptozotocin-induced oxidative stress in diabetic conditions. This has led to the generation of anti-diabetic and/or obesity treatment in humans by controlling postprandial blood-glucose levels.

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