Anti-diabetic effects of aqueous prickly lettuce (Lactuca scariola Linn.) leaves extract in alloxan- induced male diabetic rats treated with nickel (II)

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Anti-diabetic effects of aqueous prickly lettuce (*Lactuca scariola* Linn.) leaves extract in alloxan-induced male diabetic rats treated with nickel (II)

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Abstract

Background: Hattaraki pallye or prickly lettuce (*Lactuca scariola* Linn.) is one among several green leafy plants that grow in north Karnataka; it is usually consumed by the people of this region and is found to be antidiabetic in nature. The objective of this study is to evaluate hypoglycemic activities of supplementation with aqueous extract of prickly lettuce (*L. scariola*) leaves in vivo in acute and subchronic exposure with or without nickel (II) along with its glucose reduction capabilities with or without nickel (II) at pH 7.0 and 9.0 in vitro.
Methods: Percentage glucose reduction (in vitro) was determined by glucose oxidase-peroxidase enzymatic method at pH 7.0 and pH 9.0 using UV-Vis spectrophotometer. Hypoglycemic activities of *L. scariola* were carried out in alloxan-induced male diabetic rats at both acute and subchronic exposure.
Results: The results showed a significant alteration in the λ_{max} value of Ni (II) in combination with *L. scariola* leaves extracts at both pH 7.0 and 9.0. The aqueous extract also produced a significant reduction in the glucose concentration at pH 7.0 and pH 9.0 even in presence of Ni (II) in vitro. *Lactuca scariola* leaves in either acute or subchronic supplementation showed a greater glucose tolerance and hypoglycemic regulation of blood sugar in diabetic rats with or without nickel (II) treatments.
Conclusions: *Lactuca scariola* leaves can be a substitute for synthetic drugs to treat diabetic patients.

Keywords: hypoglycemia; *Lactuca scariola* Linn. extract; nickel (II); pH alteration.

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Introduction

Hattaraki pallye or prickly lettuce (*Lactuca scariola* Linn.) is among several green leafy plants that grow in north Karnataka and is consumed by the people of this region; it is found to be antidiabetic in nature. It is wildly grown in cultivated fields as a weed and is considered to have lots of medicinal values; notably, it exhibits analgesic, sedative, and diuretic properties and is also used to combat insomnia [1]. As a result, it is the most popular of all salad vegetables [2]. Heavy metals like nickel are considered one of the important industrial hazards which are found to induce diabetes mellitus among the people who are exposed to it [3]. It is found to alter insulin response. Most of the synthetic drugs which are routinely used to control diabetes seem to have many side effects; therefore, there is a need to replace these with various plant-based alternatives. Many herbal medicines that control diabetes are listed in the Ayurvedic literature [4]. As the digestive tract consists of different pH environments (acidic in stomach, alkaline in intestine and pH 7.4 in blood), the present work was undertaken to study the glucose-reduction capabilities of *L. scariola* alone and in combination with nickel (II) at two different pH conditions in vitro; also, hypoglycemic activities of *L. scariola* in alloxan-induced diabetic rats exposed to nickel (II) in vivo are carried out.

Materials and methods

Collection and identification of plant

The fresh prickly lettuce (*L. scariola*) leaves were collected locally from the market and were authenticated [5] by Dr. M.B. Mulimani, Associate Professor, and Dr. Paramanna D, Associate Professor, Department of Botany, B.L.D.E.A's S.B. Arts and K.C.P. Science College, Bijapur, Karnataka, India.

Preparation of plant extract

Fresh prickly lettuce (*L. scariola*) leaves (5 g) were washed thoroughly with distilled water, crushed and extracted with 100 mL distilled water at temperature 50–60 °C repeatedly, for 1 h. The resulting extract was filtered using Whatmann filter paper no. 1. The filtrate was used as an aqueous plant extract and orally administered to the animals by gastric intubation using a gavage during the experimental period.

Phytochemical analysis

Freshly prepared *L. scariola* extract was evaluated to find out the phytochemical constituents using standard procedures (Table 1) [6].

Spectral analysis

The chemical behavior of nickel (II) alone and in combination with *L. scariola* extracts at pH 7.0 and pH 9.0 (in vitro) were recorded using double-beam UV-visible spectrophotometer (Elico SL 210; Elico Pvt, Ltd., Bangalore, Karnataka, India).

In vitro percentage glucose reduction at different pH

The percentage glucose reduction (in vitro) was determined by glucose oxidase-peroxidase enzymatic method [7, 8] at pH 7.0 and pH 9.0. Briefly, 0.2 mL standard glucose solution (20 mg/dL) was added to 0.8 mL of triple-distilled water in a test tube followed by adding 0.5 mL of *L. scariola* extracts and 1 mL of glucose oxidase reagent to the test tube and incubated at 35 °C for about 40 min. The reaction was terminated by adding 2 mL of 6 N HCl. Results were spectrophotometrically recorded at 540 nm. Percentage glucose reduction was measured using the following equation:

$$\text{Concentration of glucose} = \frac{\text{Absorbance of glucose} + \text{Extract}}{\text{Absorbance of glucose}} \times \text{Concentration of glucose}$$

A similar procedure was followed to determine the percentage glucose reduction in combinations of nickel (II) and *L. scariola* extracts. Each of the experiments was repeated 10 times.

Animals

Adult male albino rats of Wistar strain (*Rattus norvegicus*) weighing 180–200 g were housed in central animal house of BLDE University's Shri. B.M. Patil Medical College, Hospital and Research Centre, Bijapur, with 12 h light and 12 h dark cycles. The rats were divided into nine groups with each group having six rats. Three rats were kept in each metabolic wire cage (60 cm×30 cm×20 cm). Standard pellets obtained from Hindustan Lever rat feed (Mumbai, India) were used as a basal diet during the experimental period. The control and experimental rats were provided food and drinking water ad libitum.

Chemicals

Following is the list of chemicals used: alloxan monohydrate (Spectrochem Pvt. Ltd., Mumbai, Maharashtra, India), glipizide (Sun Pharma Ltd., Mumbai, Maharashtra, India), Tween 80 (S.D. Fine-Chem Limited, Mumbai, Maharashtra, India), Accu-chek® Active Glucometer (Roche Diagnostics, Mannheim, Germany), blood gluco-strips (Roche Diagnostics, Mannheim, Germany), horseradish peroxidase (Merck Specialties Pvt. Ltd., Mumbai, Maharashtra, India), and glucose oxidase (Sigma Life Sciences 49180, St. Louis, USA). All other chemicals and reagents used were of analytical grade.

Acute oral toxicity studies of extract

The acute oral toxicity studies of extracts were carried out as per the Organization for Economic Cooperation and Development guidelines, draft guidelines 423 adopted on 17 December 2001 received from CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), Ministry of Social Justice and Empowerment, Government of India [9]. Administration of the stepwise doses of aqueous extracts of prickly lettuce (*L. scariola*) leaves from 5 mg/100 g body weight up to a dose of 12.5 mg/100 g body weight caused no considerable signs of toxicity in the tested animals. One tenth of the upper limit dose was selected as the level for examination of antidiabetic activity.

Experimental groups

The experimental rats were grouped as shown in Table 1 [10, 11].

Table 1: Experimental groups.

Groups	Supplementation	Dosage
Group I	Normal control	
Group II	Non diabetic rats+*L. scariola* extract	(12.5 mg/100 g, orally)
Group III	Non diabetic rats+nickel (II)	(2.0 mg/100 g body weight i.p.)
Group IV	Non diabetic rats+nickel (II)+*L. scariola* extract	(2.0 mg/100 g body weight; i.p.) and (12.5 mg/100 g, orally)
Group V	Diabetic control	
Group VI	Diabetic rats+*L. scariola* extract	(12.5 mg/100 g, orally)
Group VII	Diabetic rats+nickel (II)	(2.0 mg/100 g body weight; i.p.)
Group VIII	Diabetic rats+nickel (II)+*L. scariola* extract	(2.0 mg/100 g body weight; i.p.) and (12.5 mg/100 g, orally)
Group IX	Diabetic rats+antidiabetic drug, Glipizide (reference drug)	2.5 mg/kg

Induction of diabetes

The rats were made to fast overnight, and diabetes was induced with a single intraperitoneal injection (i.p.) of 15% freshly prepared solution of alloxan monohydrate (15 mg/100 g body weight) in sterile normal saline just before use. Since alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, rats were treated with 20% glucose solution (i.p.) after 6 h. The rats were then kept for the next 24 h on 5% glucose solution bottles in their cages to prevent hypoglycemia [12]. After 3 days blood samples were collected from the tail vein. Serum was separated, and fasting glucose levels were estimated by glucose oxidase-peroxidase enzymatic method [13] to confirm diabetes. Only those rats which showed hyperglycemia (glucose level >250 mg/dL) were selected for the study.

Oral glucose tolerance test

Fasting blood sugar level (FBS) was measured in the rats of all groups. Thirty minutes after administration of *L. scariola* leaves extract, or nickel (II), or both, or glipizide treatment, the rats of all nine groups (non-diabetic: groups I to IV and diabetic: groups V to IX) were orally treated with glucose at 0.35 mg/100 g body weight [14]. Blood samples were collected from the rat tail vein just prior to glucose administration (0.0 h) and at 0.5 h, 1.0 h, 1.5 h and 2.0 h after glucose loading. Blood glucose levels were measured immediately by using a glucometer.

The *L. scariola* leaves extract and glucose regulation on alloxan-induced diabetic rats for 2 weeks

After the day of oral glucose tolerance test (OGTT), administration of extracts (12.5 mg/100 g body weight, orally) was continued every day in designed experimental groups (group II, group IV, group VI and group VIII) till 13 days. Similarly, nickel (II) (2.0 mg/100 g body weight; i.p. on day 1, day 3, day 5, day 7, day 9, day 11 and day 13) was administered in group III, group IV, group VII and group VIII rats. Blood sample was collected from the rat tail vein after 30 min of either ingestion of plant extracts or nickel (II) treatment or both and glipizide treated rats on the 1st, 3rd, 5th, 7th, 9th, 11th and 13th days in the rats of all groups. Blood glucose levels were measured by using a glucometer.

Statistical analysis

The statistical data obtained from all the control and experimental samples were analyzed to evaluate the range of significance. Results expressed as mean±SD values were calculated for each group. To determine the significance of inter-group differences, one-way analysis of variance followed by post hoc t test was done.

Ethics

All the animal experiments were conducted according to the ethical norms approved by CPCSEA, Ministry of Social Justice and Empowerment, Government of India, and ethical clearance was granted by the Institutional Animal Ethical Committee, BLDE University, Bijapur.

Results

Phytochemical analysis

The preliminary phytochemical analysis of aqueous extract of prickly lettuce leaves (*L. scariola* extract) revealed the presence of flavonoids, saponins and steroids (Table 2).

Spectral analysis

The results revealed that the λ_{max} value of nickel (II) at pH 7.0 and pH 9.0 were 394.0 nm and 396.0 nm, respectively. The combination of nickel (II) with aqueous extract of *L. scariola* showed a hypsochromic shift to 330.0 nm and 306.0 nm at pH 7.0 and pH 9.0, respectively (Table 3).

In vitro percentage glucose reduction at different pH

It was noticed that the aqueous extract of *L. scariola* significantly decreased percentage glucose level at pH 7.0 and pH 9.0 with or without combination of nickel (II) in vitro as tabulated in Table 4.

Table 2: Phytochemical analysis of *L. scariola* extracts.

Sl. no.	Phytochemical constituents	Name of the test	Aqueous extract
1.	Alkaloids	Mayer's test	–
		Dragondorff's test	–
		Wager's test	–
2.	Carbohydrates	Molish's test	–
		Fehling's test	–
		Benedict's test	–
3.	Flavonoids	Lead acetate test	+
		Mineral acid test	+
4.	Saponins	Libarmann Burchard test	+
5.	Tannins	Gelatin test	–
		Ferric chloride	–
6.	Triterpenes	Libermann storch Morawski test	–
		Salkowski's test	–
7.	Steroids	Libermann Burchard sterol reaction	+
8.	Caretenoids	Antimony trichloride	–
		Conc. HCl with phenol	–

Table 3: λ_{max} and absorbance values of nickel (II) alone and in combination with *L. scariola* leaves extract at different pH.

Solution samples	Concentration of samples	pH 7.0		pH 9.0	
		λ_{max}, nm	Absorbance	λ_{max}, nm	Absorbance
Nickel (II)	6 mg/mL	394.0[a]	0.1329	396.0	0.1274
Nickel (II)+extract	6 mg/mL+0.5 mL of extract	330.0	4.3087	306.0	4.0569

Extract: *Lactuca scariola* leaves extract; [a]Data shown as λ_{max} and absorbance values by nickel (II) alone and in combination with extract by using UV-VIS Spectrophotometer at pH 7.0 and 9.0.

Table 4: In vitro hypoglycemic effect of *L. scariola* leaves extract alone and in combination with nickel (II) at different pH.

S. no.	Solution samples	pH 7.0	pH 9.0
1.	Glucose+extract	68.6%[a]	97.6%
2.	Glucose+extract+nickel (II)	47.3%	69.75%

Extract: *Lactuca scariola* leaves extract; [a]Data are shown as percent reduction of glucose concentrations in vitro by extract alone and in combination with nickel (II) using glucose oxidase-peroxidase method at pH 7.0 and 9.0.

Acute oral toxicity studies

In acute toxicity study, aqueous extracts of *L. scariola* leaves showed non-significant toxicity sign when observed for the parameters during the first 4 h and followed by daily observations for 14 days, and mortality was also not observed; the drug was found to be safe at the tested dose level of 12.5 mg/100 g body weight. One tenth of this dose level was taken as effective dose. All the extracts were tested at the same dose of 12.5 mg/100 g body weight. In order to ascertain a scientific base for the usefulness of this plant in the treatment of diabetes, it was decided to evaluate the experimental design for antidiabetic activity by following glucose tolerance test and in vitro glucose reduction.

Oral glucose tolerance test

The effect of *L. scariola* extracts on glucose tolerance test in non-diabetic and diabetic rats are shown in Tables 5 and 6, respectively. At 1.0 h after glucose administration in normal rats (group I), the peak of blood glucose level increased rapidly from the fasting value. Subsequently, the values began to decline during the next 60 min to reach its near-baseline value after 2 s of glucose administration. The *L. scariola* extract-treated non-diabetic rats (group II) showed similar results as the normal rats (group I). The blood glucose levels of extract-treated non-diabetic rats insignificantly decreased compared to normal rats at all time intervals after glucose administration. Interestingly, nickel (II)-treated group III rats showed a diabetic-like OGTT result with its peak glucose concentration at 1 h, and even at 2 h glucose concentration did not return to baseline. In the case of group IV rats (nickel (II)+extract), blood glucose concentration showed a remarkable improvement

Table 5: Effect of acute exposure to nickel (II) (2.0 mg/100 g body weight) and simultaneous supplementation with *L. scariola* leaves extract (12.5 mg/100 g body weight) on blood glucose level in normal rats.

		Non-diabetic model				
		Period after glucose ingestion, h				
Treatment groups	FBS	0.0	0.5	1.0	1.5	2.0
I	90.00±3.22^{a,1}	89.50±2.42^{a,1}	117.33±5.00^{b,1}	128.66±6.28^{c,1}	113.33±2.87^{b,1}	94.5±6.47^{a,1}
II	90.83±2.56^{a,1}	89.5±3.67^{a,1}	101.33±6.97^{b,2}	116.16±8.49^{c,2}	100.83±5.87^{b,2}	90.66±2.50^{a,1}
III	88.66±3.66^{a,1}	101.16±5.30^{b,1}	177.50±23.18^{c,3}	185.00±23.66^{c,3}	152.50±21.15^{d,3}	121.66±15.70^{e,2}
IV	89.66±4.76^{a,1}	95.33±4.08^{a,2}	113.83±3.97^{b,1}	138.83±4.95^{c,4}	125.16±6.79^{d,4}	116.66±7.44^{b,2}

Treatment groups: I, normal control; II, *Lactuca scariola* leaves extract; III, nickel (II); IV, nickel (II)+*L. scariola* leaves extract. Horizontal values are the mean±SD of six observations in each group till at 2 h. In each row, values with different superscript letters (a, b, c or d) are significantly different from each other ($p<0.05$) in the same group till 2 h. Vertical columns indicate variation of blood glucose levels among four different groups at different time interval till 2 h. In each column, values with different superscript numbers (1, 2, 3 or 4) are significantly different from each other ($p<0.05$) at different times.

Table 6: Effect of acute exposure to nickel (II) (2.0 mg/100 g body weight) and simultaneous supplementation with *L. scariola* leaves extract (12.5 mg/100 g body weight) on blood glucose level in diabetic rats.

				Diabetic model		
				Period after glucose ingestion, h		
Treatment groups	FBS	0.0	0.5	1.0	1.5	2.0
V	257.66±10.59^{a,1}	257.5±10.36^{a,1}	322.83±14.64^{b,1}	395.33±7.39^{c,1}	375.66±3.82^{d,1}	350.83±7.35^{e,1}
VI	255.33±8.16^{a,1}	234.50±88.91^{b,2}	286.83±8.63^{c,2}	350.66±12.37^{d,2}	322.5±11.36^{e,2}	268.50±10.74^{f,2}
VII	252.66±10.19^{a,1}	278.50±12.62^{b,3}	381.00±12.48^{c,3}	402.00±12.40^{d,1}	415.66±14.05^{d,3}	405.00±14.17^{d,3}
VIII	256.00±8.48^{a,1}	263.00±99.6^{a,1}	324.00±7.12^{b,1}	382.66±10.98^{c,3}	381.33±9.72^{c,1}	368.00±6.81^{d,4}
IX	251.83±5.74^{a,1}	236.33±11.91^{b,2}	275.00±5.79^{c,2}	296.50±4.37^{d,4}	216.66±10.63^{e,4}	154.16±20.35^{f,5}

Treatment groups: V, diabetic control; VI, diabetic+*L. scariola* leaves extract; VII, diabetic+nickel (II); VIII, diabetic+nickel (II)+*L. scariola* leaves extract; IX, diabetic+glipizide (0.25 mg/100 g). Horizontal values are the mean±SD of six observations in each group till at 2 h. In each row, values with different superscript letters (a, b, c or d) are significantly different from each other ($p<0.05$) in the same group till 2 h. Vertical columns indicate variation of blood glucose levels among five different groups at different time interval till 2 h. In each column, values with different superscript numbers (1, 2, 3 or 4) are significantly different from each other ($p<0.05$) at different times.

in OGTT (Table 5). In the case of group V (diabetic control) rats, the peak glucose concentration in OGTT showed at 1 h, and even at 2 h it showed typical diabetic response, whereas in group VI (diabetic+*L. scariola* extract), rats showed declined blood glucose levels at all the time intervals and almost reached its baseline at 2 h. In the case of group VII (diabetic+nickel (II)), a greater diabetic OGTT response as compared to diabetic alone was noticed at all the given times, whereas in group VIII (diabetic+nickel (II)+*L. scariola* extract), rats showed improved diabetic response by reducing glucose concentration in OGTT at all the given times as compared to group VII. Further, we found that glipizide (as a reference drug) produced a fall of blood glucose levels at all the time intervals after glucose administration in group IX (diabetic+glipizide) rats (Table 6). The results of each group were significantly different at different time intervals from each other ($p<0.05$).

Subchronic effects (13 days) of *L. scariola* leaves extract and glucose regulation on alloxan-induced diabetic rats

Group III (non diabetic+nickel (II)) rats showed clear hyperglycemia as compared to the non-diabetic control rats (group I). The *L. scariola* extract significantly lowered elevated blood glucose levels in group IV (nickel (II)+*L. scariola* extract) as compared to only nickel (II)-treated non-diabetic (group III) rats (Table 7). It was also noticed that there was a progressive hypoglycemic effect of extracts in group IV rats till the 13th day. Diabetic nickel (II)-treated group VII rats showed progressive rise of blood glucose concentration till the 13th day, but in group VIII (diabetic+nickel (II)+*L. scariola* extract), remarkable progressive reduction in glucose concentrations was noticed. Group VIII rats also showed a reduction of blood glucose

Table 7: Effect of subchronic treatment with nickel (II) (2.0 mg/100 g body weight) and simultaneous supplementation with *L. scariola* leaves extract (12.5 mg/100 g body weight) on blood glucose level in normal rats.

				Non-diabetic model			
				Blood glucose levels, mg/dL			
Treatment groups	1st day	3rd day	5th day	7th day	9th day	11th day	13th day
I	89.33±2.58^{a,1}	90.50±2.50^{a,1}	91.83±1.94^{a,1}	90.66±1.75^{a,1}	89.83±1.32^{a,1}	89.66±1.03^{a,1}	91.00±0.89^{a,1}
II	90.83±2.48^{a,1}	89.33±2.33^{a,1}	86.66±1.86^{a,1}	87.16±3.06^{a,1}	90.00±2.44^{a,1}	89.16±2.63^{a,1}	87.66±2.73^{a,1}
III	88.66±3.66^{a,1}	112.5±8.80^{b,2}	134.16±7.22^{c,2}	137.50±7.03^{c,2}	138.50±6.09^{c,2}	140.66±5.78^{d,2}	142.16±5.34^{d,2}
IV	89.83±4.16^{a,1}	106.50±6.50^{b,2}	116.83±2.99^{c,3}	120.66±3.76^{c,3}	122.16±3.76^{c,3}	120.00±4.42^{c,3}	116.83±2.71^{c,3}

Treatment groups: I, normal control; II, *Lactuca scariola* leaves extract; III, nickel (II); IV, nickel (II)+*L. scariola* leaves extract. Horizontal values are the mean±SD of six observations in each group in every alternate day till 13th day. In each row, values with different superscript letters (a, b, c or d) are significantly different from each other ($p<0.05$) in the same group at different days. Vertical columns indicate variation of blood glucose levels among four different groups in each alternate day till 13th day. In each column, values with different superscript letters (1, 2, 3 or 4) are significantly different from each other ($p<0.05$) at different days.

concentration on the 13th day which is actually below the baseline value from the 1st day (Table 8). The reference drug glipizide lowered the blood glucose level in diabetic rats (group IX) significantly, bringing it nearly back to the normal. The values are significantly different from each other ($p<0.05$) in the same group at different days.

Discussion

Our results reflect alteration of chemical behavior of nickel (II) in combination with aqueous *L. scariola* extracts at both pH 7.0 and pH 9.0 in vitro, which indicates that the aqueous *L. scariola* extract influences the change of chemical behavior of nickel (II) irrespective of the pH of the environment, possibly by molecular interaction between nickel (II) and the antioxidant constituent of phytochemicals [15]. It can be seen from the observations of Table 3 that aqueous *L. scariola* extracts reduced greater glucose concentration in alkaline pH 9.0 as compared to neutral pH 7.0 in vitro. Similarly, it was noticed that in the presence of nickel (II) supplementation in vitro, percentage reduction of glucose concentration is also greater at pH 9.0 as compared to pH 7.0. Hence, it can be postulated from our study that pH of the environment influences glucose reduction capabilities of aqueous *L. scariola* extracts with or without the presence of heavy metals like nickel (II). The results of OGTT and chronic blood glucose levels clearly indicate that *L. scariola* extract showed hypoglycemic activity. Blood glucose level is commonly measured to check glycemic control mechanism. In the present study, *L. scariola* extract has shown significant reduction in the blood glucose level. The results were significant with ($p<0.05$) with different groups at different time intervals and different days. This could be due to increase in the pancreatic secretion of insulin by the β cells of islets of Langerhans. It is well known that flavonoids and saponins are bioactive antidiabetic components. The phytochemical screening of aqueous *L. scariola* extracts revealed the presence of these bioactive components. Flavonoids act on various molecular targets and regulate different signaling pathways in pancreatic β cells [16]. Perhaps saponin influences structural alterations of glucose molecule through glycone and glucuronic acid [17]. Hence, it can be predicted that the hypoglycemic activities of *L. scariola* extracts may be due to the effect of these bioactive components. Nickel (II) induces hyperglycemia in both acute and subchronic exposure conditions, which could be due to an increased pancreatic release of glucagons [18, 19]. Such alterations were found to lead to a drastic drop in the insulin/glucagon plasma ratio [20]. Another explanation for the nickel-induced rise of blood glucose level in male rats could be an involvement of nitric oxide-mediated pathways [21]. Nickel causes an increase in the level of cyclic guanosine monophosphate and constitutive nitric oxide synthase in the adrenals and brain or inducible nitric oxide synthase in the pancreas by modulating the release of insulin from pancreas, finally leading to hyperglycemic condition. In our study, we found a significant improvement in blood glucose levels in diabetic rats treated with both nickel (II) and *L. scariola* when compared to only nickel-treated diabetic rats in acute (OGTT) or subchronic conditions. This finding may be due to an improvement of nickel-induced

Table 8: Effect of subchronic treatment with nickel (II) (2.0 mg/100 g body weight) and simultaneous supplementation with *L. scariola* leaves extract (12.5 mg/100 g body weight) on blood glucose level in diabetic rats.

							Diabetic model
							Blood glucose levels, mg/dL
Treatment groups	**1st day**	**3rd day**	**5th day**	**7th day**	**9th day**	**11th day**	**13th day**
V	$253.16\pm7.22^{a,1}$	$262.66\pm5.57^{a,1}$	$274.00\pm9.87^{b,1}$	$295.66\pm8.04^{c,1}$	$298.00\pm10.65^{c,1}$	$312.33\pm6.37^{d,1}$	$326.66\pm7.25^{d,1}$
VI	$252.50\pm10.32^{a,1}$	$235.16\pm6.33^{b,2}$	$219.83\pm4.53^{c,2}$	$196.00\pm4.89^{d,2}$	$178.33\pm8.16^{e,2}$	$157.50\pm8.75^{f,2}$	$141.33\pm9.45^{g,2}$
VII	$251.33\pm7.55^{a,1}$	$266.33\pm6.53^{b,1}$	$272.66\pm6.12^{b,1}$	$293.00\pm4.14^{c,1}$	$296.33\pm3.88^{c,1}$	$311.83\pm7.49^{c,1}$	$326.83\pm2.48^{d,1}$
VIII	$256.00\pm8.48^{a,1}$	$260.33\pm9.09^{a,1}$	$248.16\pm5.67^{b,3}$	$238.00\pm6.75^{b,3}$	$227.50\pm7.55^{c,3}$	$215.66\pm6.77^{d,3}$	$169.00\pm7.94^{e,3}$
IX	$254.56\pm9.45^{a,1}$	$250.60\pm10.37^{a,3}$	$175.28\pm9.87^{b,4}$	$142.49\pm10.05^{c,4}$	$125.28\pm9.11^{d,4}$	$109.43\pm10.28^{e,4}$	$94.24\pm9.26^{f,4}$

Treatment groups: V, diabetic control; VI, diabetic+*L. scariola* leaves extract; VII, diabetic+nickel (II); VIII, diabetic+nickel (II)+*L. scariola* leaves extract; IX, diabetic+glipizide (0.25 mg/100 g). Horizontal values are the mean±SD of six observations in each group in every alternate day till 13th day. In each row, values with different superscript letters (a, b, c, d, e, f or g) are significantly different from each other ($p<0.05$) in the same group at different days. Vertical columns indicate variation of blood glucose levels among five different groups in each alternate day till 13th day. In each column, values with different superscript numbers (1, 2, 3 or 4) are significantly different from each other ($p<0.05$) at different days.

oxidative stress and insulin resistance by *L. scariola*. Our study revealed a relationship between *L. scariola* and glucose metabolism, as there was increase in insulin sensitivity in diabetic rats treated with *L. scariola* in both acute and subchronic conditions. Possibilities of decreased glucose uptake by gut due to *L. scariola* extract supplementation might not be ruled out. In our study, diabetic rats treated with nickel (II) did not show additional hyperglycemic response as compared to diabetic rats alone. On the other hand, supplementation with *L. scariola* to nickel (II)-treated diabetic rats showed decreased blood glucose level in both OGTT and subchronic exposures. It may be possible that the decrease of nickel-induced oxidative stress and insulin resistance by *L. scariola* with concomitant increase of insulin sensitivity are the causes of this.

Conclusions

In vitro and in vivo results obtained from this study reveal that *L. scariola* leaves have significant hypoglycemic and antidiabetic potential. The *L. scariola* leaves may be used as a substitute for synthetic drugs to treat diabetic patients. The present study has also opened avenues for further research, especially with reference to the different dose studies and development of potent formulation for diabetes mellitus from *L. scariola* leaves.

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