Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(2): 1423-1426
Received: 06-01-2019
Accepted: 09-02-2019

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Antihyperglycemic effect of leaves and inflorescences of *Girardinia heterophylla* on Streptozotocin-nicotinamide induced type-II diabetic male albino wistar rats

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Abstract
Leaf and inflorescence of *Girardinia heterophylla* is believed to be beneficial for diabetic patients by the local community of Sikkim, thus its degree of use is very high. Present study evaluated the antihyperglycemic activity of hydroethanolic extract of these two plant parts. Preliminary phytochemical analysis revealed number of phytocompounds. Streptozotocin-nicotinamide induced Type-II diabetic male albino Wistar rats were orally fed with plant extracts (200 and 400 mg/kg body weight) for 28 consecutive days. There was significant (p<0.05) dose dependent decrease in the blood glucose level in the experimental group as compared to the diabetic control group (percentage reduction of 37.29% and 45.54% by leaf extract, 65.46% and 66.67% by inflorescence extract at two different concentrations). Normalization of body weight was also observed. Results of the present study strongly indicate that leaves and inflorescences of *Girardinia heterophylla* have antihyperglycemic potential and can be source for the isolation of active compound(s).

Keywords: Hydroethanolic extract, antihyperglycemic, streptozotocin-nicotinamide

Introduction
Diabetes mellitus, an endocrine disorder, is a leading cause of morbidity and mortality in both developing and developed countries [1]. To treat diabetes and its complications, numbers of synthetic drugs are available but the lifestyle modification, change in food habits and use of medicinal plants have more beneficial effects [2, 3]. Thus, the research for possible antihyperglycemic and antidiabetic activities of plants is expanding around the globe [4].
Girardinia heterophylla belonging to the Family Urticaceae is found in temperate and sub-tropical Himalayas. It can be seen growing extensively in the banks of streams in the moist forest areas at an elevation approximately between 1,200 to 3,000 metres [5, 6]. Plant grows 8-12 feet in height and has persistent stipules and stinging trichomes [6]. The leaf and inflorescence are edible and are also believed to have antidiabetic property, as a result its degree of use is very high among the local population. Roots and rhizomes of *Girardinia heterophylla* have been used in the Indian systems of medicine and folklore medicine for number of aliments [7]. Thus, *Girardinia heterophylla* has an important association with the ethnic communities due to its unique medicinal properties [7].
Pharmacological studies revealed active constituents responsible for versatile properties of different parts including antihyperglycemic activity of root and leaf extracts, [8, 9] but the inflorescence has not been scientifically investigated. Various studies suggest that secondary metabolite concentration in the plants varies with environmental stress and climatic conditions [10, 11]. Therefore the present study intends to investigate the antihyperglycemic property of leaves and inflorescences of *Girardinia heterophylla* (LGH and IGH respectively) growing at higher elevations of foothills of Sikkim Himalaya.

Materials and Methods
The plant material collected from higher reaches of Sokpey (altitude: 3000 m - 3300 m), South District of Sikkim was identified by the Taxonomist of Department of Botany, University of North Bengal, Siliguri, India and the herbarium (accession number: 09810) was deposited. Chemicals used in this study were purchased from Sigma-Aldrich and Merck Germany. The young LGH and IGH were shed dried for 30 days and powdered (100 g each). Hydroethanol at the ratio of 70:30 (water: ethanol) was used for extract preparation using soxhlet apparatus [12]. The solvent was evaporated using rotary evaporator at 45 ºC (IKA Germany, RV 3 V) to produce a semisolid mass (28.6 g LGH and 23.3 g IGH) and was stored at 4 ºC for further use.

Phytochemical tests were performed by standard methods for the detection of phytoconstituents [13].

Experiment on male albino Wistar rats was conducted in accordance with the guidelines of the CPCSEA. The experimental protocol was approved by the Institutional Animal Ethics Committee of the Sikkim Manipal Institute of Medical Sciences (SMIMS), Gangtok, Sikkim, India (MC/SMIMS/IAEC/05/2016). Rats were housed in the animal house of SMIMS and were fed a standard rat pellet diet and water. The extract of IGH and LGH was administered orally at different dose levels of 200 mg/kg, 400 mg/kg, 600 mg/kg, 1200 mg/kg, and 2000 mg/kg of body weight in order to carry out acute oral toxicity study according to OECD guideline [14]. After administration, the rats were observed for 0, 0.5, 1.0, 24, 48, and 72h for lethal effects.

Experimental design

Male albino Wistar rats weighing 150-200 g were assigned into five groups with six animals in each group. Group I, Normal control (Served as untreated normal rats), Group II, Diabetic control (Diabetic animals fed with normal diet), Group III, Positive control (Animals treated with Glibenclamide), Group IVA and IVB, Test group (Animals treated with the plant extract at the dose of 200 mg/kg b.w. and 400 mg/kg b.w. respectively). Glibenclamide was orally administered at a dose of 0.5 mg/kg b.w. Extracts in two different concentration was fed once daily for 28 days [15]. Blood glucose level was estimated on day 0, 7th, 14th, 21st and 28th day [16]. Type-II diabetes was induced by intraperitonial administration of STZ-NA (60 mg/kg b.w) reconstituted in freshly prepared cold citrate buffer (pH 7.4) [15]. Blood samples were collected from tail vein after 72 h of STZ-NA administration and the glucose levels were measured. Rats with fasting glucose levels of ≥200 mg/dl were selected for experiment. After 72 h of STZ-NA injection extract was orally administered, once daily for the period of 28 days. In overnight fasted Wistar rats, blood samples were collected by tail vein puncture. Glucose level was estimated using One Touch (Verio Flex) glucometer [17]. Statistical analysis was carried out by using analysis of variance (ANOVA) followed by Bonferroni post tests and Dunnett's multiple comparison tests, for which Graph pad prism statistical software was used. A value of $p<0.05$ was considered to be significant.

Results

The hydroethanolic extracts of LGH and IGH were subjected to preliminary phytochemical screening. Both extracts indicated the presence of phenol, flavonoid, tannin, alkaloid, glycoside and anthocyanin. The IGH, however, did not contain saponin. Oral administration of LGH and IGH extract at different test doses did not produce toxicological effect or drug-induced harmful physical signs in any of the Wistar rats and no mortality were observed (LD_{50} >2000 mg/kg b.w.).

The effect of hydroethanolic extracts of LGH and IGH at two different doses and reference drug glibenclamide on blood glucose level of diabetic rats is depicted in Table 1 and Table 2 respectively. As compared to the diabetic control group, there was a significant ($p<0.05$) dose dependent decrease in the blood glucose level estimated on 14th, 21st and 28th day in the experimental groups treated with the extract at the concentrations of 200 mg/kg b.w. and 400 mg/kg b.w of LGH and IGH extract. Experimental animals treated with glibenclamide exhibited significant ($p<0.05$) decrease in the blood glucose level after 7 days of treatment.

Table 1: Effect of hydroethanolic extract of LGH on blood glucose levels in STZ-NA induced diabetic rats

Groups	Fasting blood glucose levels (mg/dl)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Group I	86.5±6.60	90.75±4.19	90.75±3.5	93.5±5.19	94.75±10.21
Group II	300.5±6.85	302.5±7	354.75±5.90	358.25±2.87	374.5±7.18
Group III	312.5±4.72	237±11.74*	118±5.59*	99.25±7.84*	90.75±7.04*
Group IVA	309.75±7.54	302.5±4.43	296.75±4.57*	249.5±9.03*	194.25±3.68*
Group IVB	314.5±5.06	300±2.16	272.75±5.90*	220±8.36*	171.22±2.44*

Data represents mean ± SD (n=6). * Significant ($p<0.05$) decrease in blood glucose level, when groups III, IVA and IVB were compared with group II (diabetic control group).

Table 2: Effect of hydroethanolic extract of IGH on blood glucose levels in STZ-NA induced diabetic rats

Groups	Fasting blood glucose levels (mg/dl)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Group I	77.5±5.30	81.45±5.16	86.25±3.6	94.6±5.11	94.88±9.11
Group II	288.5±6.85	297.5±2.28	324.64±4.80	361.21±5.16	369.1±3.31
Group III	292.5±4.32	266±6.79*	112±5.69*	93.55±9.34*	86.64±5.04*
Group IVA	310.53±8.34	296.4±6.33	243.63±5.37*	129.5±5.13*	107.25±7.08*
Group IVB	306.5±5.06	280±3.11	232.63±4.08*	118±6.33*	102.18±5.34*

Data represents mean ± SD (n=6). * Significant ($p<0.05$) decrease in blood glucose level, when groups III, IVA and IVB were compared with group II (diabetic control group).

LGH and IGH extract significantly increased the body weights in diabetic rats as compared to diabetic control rats as depicted in Table 3 and Table 4 respectively. Prior to STZ administration, there were no significant differences in the average body weights of all the five groups of experimental animals. By the end of the first week after diabetes mellitus was experimentally induced, the weights of groups II, III, IVA, and IVB animals were significantly decreased. Weight loss continued for four weeks in diabetic control animals. Administration of LGH, IGH extracts (200 mg/kg b.w. and 400 mg/kg b.w.) and glibenclamide significantly increased the body weights in diabetic rats after 14 days as compared to diabetic control rats (Table 3 and Table 4).

Table 3: Effect of hydroethanolic extract of LGH on body weight in STZ-NA induced diabetic rats

Groups	Average Body Weight (g)				
	Day0	Day7	Day14	Day21	Day28
Group I	173.00±4.69	177.25±3.65	179.50±3.80	180.00±4.24	182.00±3.55
Group II	166.25±3.77	148.75±3.70	145.75±1.96	139.75±1.79	133.25±2.30
Group III	178.50±3.10	163.75±3.86	170.75±1.70*	172.50±3.02*	176.00±3.65*
Group IVA	168.75±3.25	150.75±4.03	152.00±3.64*	157.50±3.68*	160.25±2.60*
Group IVB	172.25±1.50	156.00±4.32	159.50±3.31*	165.75±3.30*	169.50±4.50*

*Significant (p<0.05) increase in body weight as compared to diabetic control animals (Group II).

Table 4: Effect of hydroethanolic extract of IGH on body weight in STZ-NA induced diabetic rats

Groups	Average Body Weight (g)				
	Day0	Day7	Day14	Day21	Day28
Group I	189.00±5.59	190.55±4.32	193.00±4.66	193.15±5.46	194.10±3.25
Group II	186.22±3.17	157.75±7.10	139.34±3.89	124.45±2.69	121.85±4.01
Group III	173.78±3.10	154.66±4.81	159.95±2.71*	166.40±3.22*	170.00±2.32*
Group IVA	181.33±3.29	161.35±4.13	165.11±3.64*	172.30±5.88*	176.21±2.22*
Group IVB	176.15±2.52	152.10±4.32	158.31±4.26*	163.65±4.21*	171.72±4.68*

*Significant (p<0.05) increase in body weight as compared to diabetic control animals (Group II).

Discussion

In the present study the hydroethanolic extract of LGH and IGH at the two different concentrations exhibited antihyperglycemic activity. Various scientific study reported the presence of active constituents such as β-sitosterol, γ-sitosterol and ursolic acid isolated from the roots extract of *Girardinia heterophylla* [18]. β-sitosterol and γ-sitosterol has been reported to possess hypoglycemic and antidiabetic activities [19-22]. The compound β-sitosterol has been reported in the LGH as well [23]. Presence of this compound along with other phytoconstituents could be responsible for its antihyperglycemic activity observed in our study.

The LGH in the present study as compared to that of previous report [8] showed antihyperglycemic activity with slight difference in the potency. The habitat of the plant from which the leaf samples were collected may have influenced the active constituent. IGH on investigation has been found to be more effective as compared to the LGH. There is the possibility of greater concentration of the active constituents in the IGH. However further investigations are required to identify the active constituent including compounds like sitosterol or ursolic acid if present at varied concentration in different plant parts accountable for antihyperglycemic activity.

Hydroethanolic extract of LGH and IGH also showed marked effect in controlling the loss of body weight of diabetic rats. This may be the result of improvement of hyperglycemia. Hence, lowering of glucose levels may have prevented lipolysis of stored fat and degradation of muscle protein which or else are utilized in diabetic rats, there by normalizing the body weight.

Conclusion

The present study indicated that the treatment with hydroethanolic extract showed antihyperglycemic effect as well as improved body weight in STZ-NA induced diabetic rats. The extract of IGH showed better glycemic control as compared to LGH. The phytochemical analysis revealed the presence of various phytocompounds which could be responsible for its activity. Hence, the extract of IGH as well as LGH can be explored further for therapeutic purpose.

Acknowledgements

Authors are thankful to Department of Physiology, Department of Pharmacology and Department of Biochemistry, Sikkim Manipal Institute of Medical Sciences for providing necessary laboratory facilities for this research work.

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