ANTI-DIABETIC ACTIVITY OF INDIAN *Hypericum perforatum* L. ON ALLOXAN-INDUCED DIABETIC RATS

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Summary

Anti-diabetic activity of the standardised extract of Indian *Hypericum perforatum* L. was investigated on alloxan induced diabetic rats. Indian *Hypericum perforatum* (IHp) extract was orally administered (100 and 200 mg/kg) as suspension in 0.3 % carboxy methyl cellulose for 14 days. Glibenclamide (10 mg/kg/day, p.o.) was used as standard. Blood samples were collected at day 0 and 14th day (1 hour after last dose) from orbital sinus and blood glucose was estimated by commercially available kit. IHp treatment led to significant fall ($p<0.01$) in elevated blood glucose level. Moreover, IHp treatment also reverses the weight loss associated with alloxan treatment. The overall results indicate that IHp possess significant antidiabetic activity.

Key words: Hypoglycemic, Diabetes, Alloxan, *Hypericum perforatum*.

Introduction

For a long time, diabetes has been treated with several medicinal plants or their extracts. Before the discovery of insulin in 1922, the only treatment options for diabetes were those based on traditional practices [1]. Ethnobotanical knowledge played a particularly important role in historical diabetes therapies, with over 1200 species of medicinal plants recognized throughout the world for their ability to treat diabetic indications. Recently, attention to natural products has increased once again, but there is a need for thoroughly controlled studies on the effectiveness and potential risks of treatment with such products [2].

Hypericum perforatum also called as St. John's wort (SJW) is distributed in Europe, Asia, North Africa and North America. Indian *Hypericum perforatum* (IHp) is a rhizomatous perennial herb growing up to a height of 3 feet distributed in the western Himalayas at altitudes of 3000-10,500 feet [3]. *Hypericum perforatum* has been known for a long time for its putative medicinal properties including wound-healing [4], anti-inflammatory [5, 6], diuretic, antibiotic and antiviral [7, 8], antidepressant [9-11] and nootropic activity [12].

Principle constituents reported from SJW include the napthodianthrones hypericin and pseudohypericin, a broad range of flavonoids, including quercetin, quercitrin, amentoflavone and hyperin, the phloroglucinols hyperforin and adhyperforin, essential oils and xanthones [13, 14]. Flavonoids like quercetin have proved antidaibetic potential [15, 16]. Recent report suggests that SJW extract and hyperforin target key mechanisms of cytokine-induced β-cell injury, thereby improving β-cell function and survival. Therefore, potentially valuable for the prevention or limitation of β-cell loss in diabetes [17]. Keeping this background in mind and the fact that SJW had proven efficacy on various neurological problems like depression, anxiety and memory deficit (which are often associated with diabetes) it seems pragmatic to evaluate IHp for putative anti-diabetic activity.

Materials and Methods

Animals: Charles foster rats of either sex (body weight: 180 ± 10 g) were obtained from Central Animal House of Institute of Medical Sciences, Banaras Hindu University, Varanasi. All animals were allowed free access to tap water and pellet diet and maintained at room temperature in plastic cages.

Drugs and Chemicals: The standardised extract (50% ethanolic extract; standardised for 4.5-5% hyperforin, HPLC) of Indian *Hypericum perforatum* (IHp) was obtained from Indian Herbs Research & Supply Co. Ltd., Saharanpur, UP. Alloxan was procured from S D Fine-Chem, India and glibenclamide was a gift sample from Cipla Pharma, India.

Experimental Method: Diabetes was induced chemically by alloxan. Rats were injected 120 mg/kg body weight alloxan intra-peritonially after overnight fasting. These animals were kept for a week to stabilize the diabetic condition. Animals showing fasting blood glucose levels more than 200 mg/dl were considered as diabetic [18-20] and used for the study. Animals were divided into five groups, each containing six rats as Group I (normal control), Group II (diabetic control), Group III (diabetic rats treated with glibenclamide, 10 mg/kg/day, p.o.), Group IV (diabetic rats treated with IHp 100 mg/kg/day, p.o.) and Group V (diabetic rats treated with IHp 200 mg/kg/day, p.o.).

IHp extract was orally administered as suspension in 0.3 % carboxy methyl cellulose (CMC) for 14 days. Animals of Group I & II were given equal volume of vehicle (0.3% CMC suspension). Blood samples were collected at 0 and 14th day (1 hour after last dose) from orbital sinus and blood glucose was estimated by commercially available kit (Span Diagnostics Ltd., Surat, India). Body weight of rats was measured before and after the treatment.

Statistical analysis

All the values of the experimental results were expressed as mean ± Standard Error of Mean (SEM). Statistical analyses were performed by one way analysis of variance (ANOVA) followed by Dunnett Multiple Comparisons Test. Student's *t*-test was used for comparison within the same group before and after treatment. GraphPad InStat (version 3.06) software was used for all statistical analyses.

Results

Effect on blood glucose: The fasting blood glucose levels of diabetic control rats (Group II) were significantly higher than those of normal control rats (Group I). There was a significant fall in blood glucose level ($p<0.01$, compared to diabetic control) in both IHp treated groups (100 mg/kg as well as 200 mg/kg, p.o.). In the untreated animals (Group II), blood glucose levels did not change significantly throughout the study period (Table-1). There was significant ($p<0.05$ and $p<0.001$) fall in blood glucose level in IHp (200 mg/kg) and glibenclamide treated rats (Figure-1).

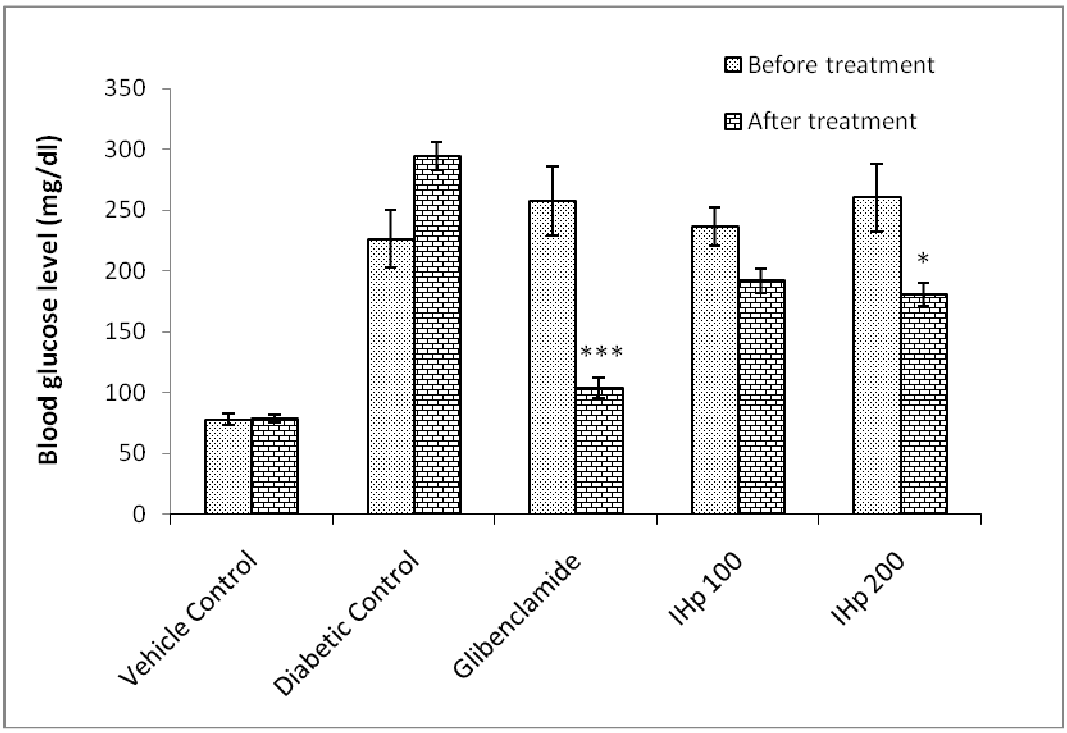
Effect on body weight: There was a significant loss in body weight in diabetic control animals (Group II). Alloxan induced body weight loss was reversed significantly by glibenclamide 10 mg/kg ($p<0.01$) and IHp 200 mg/kg ($p<0.001$), whereas IHp (100 mg/kg) has not shown such effect significantly (Table-1).

Table-1: Effect of IHp treatment on blood glucose level and body weight

Group	**Fasting blood glucose level (mg/dl)**		**Body weight (g)**	
	0 day	**14th day**	**0 day**	**14th day**
Normal control	77.95 ± 4.4	78.53 ± 3.4	177 ± 1.87	186 ± 1.82
Diabetic control	226.12 ± 23.88	294.04 ± 11.66	178.8 ± 1.92	168.6 ± 2.6#
Diabetic + glibenclamide	257.55 ± 28.09	103.5 ± 8.52**	177.4 ± 2.56	183.2±2.92††
Diabetic + IHp 100	236.25 ± 16.05	192.0 ±10.14**	175.8 ± 2.46	177.6 ± 1.80
Diabetic + IHp 200	260.33 ± 27.62	180.4 ± 9.33**	180 ± 3.08	187.2±2.3†††

Values are means ± SEM (n=6)
** = $p<0.01$, compared to diabetic control
# = $p<0.001$, compared to normal control
†† = $p<0.01$, ††† = $p<0.001$, compared to diabetic control

Figure-1: Intra-group comparison of blood glucose level before and after treatment



Values are means ± SEM (n=6); * = $p<0.05$, *** = $p<0.001$

Discussion

The present study was undertaken to study the antidiabetic activity of IHp in rats in order to scientifically validate IHp in the treatment of diabetes.

Administration of alloxan (120 mg/kg, i.p.) led to about 3-fold elevation of fasting blood glucose levels, which was maintained over a period of 3 weeks. Two weeks of daily treatment with IHp caused a significant fall in elevated blood glucose levels. Vehicle control animals were found to be stable in their body weight but diabetic rats showed significant reduction in body weight on 14^{th} day (Table-1).

It is well established that alloxan administration to experimental rats selectively causes pancreatic β cell-membrane disruption and cytotoxicity after its intracellular accumulation [21]. The antihyperglycemic activity caused by glibenclamide in alloxan-induced diabetic rats is an indication of the presence of some β cells, as glibenclamide is known to stimulate insulin secretion from β cells. The standardised extract of IHp (50% ethanolic extract; standardised for 4.5-5% hyperforin, HPLC) may have stimulating effect on the remnant β cells. Increased peripheral utilization of glucose may also be the likely mechanism of blood glucose lowering effect of IHp extract.

In a recent *in vitro* study, hyperforin was shown to have protective effect against cytokines induced pancreatic β cell damage in INS-1E β cell line [17], claiming potentially valuable for prevention or limitation of β cell loss in diabetes. Hyperforin was shown to inhibit the cytokines induced activation of nuclear-factor-kappa B (NF-kB), signal-transducer-and-activator-of-transcription-1 (STAT-1) and inducible nitric-oxide-synthase (iNOS) [17]. However, no *in vivo* study has been reported so far for antidiabetic action of hyperforin or hyperforin enriched standardised extract as we have used in the study.

Conclusion

We report for the first time *in vivo* anti-diabetic activity of standardised extract of Indian *Hypericum perforatum* which might be potentially valuable for the treatment of diabetes.

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References

1. Marles RJ, Farnsworth NR. Antidiabetic plants and their active constituents. Phytomedicine 1995; 2:13–189.
2. Frode TS, Medeiros YS. Animal models to test drugs with potential antidiabetic activity. J Ethnopharmacol 2008; 115:173–183.
3. Sastri BN (Editor). The Wealth of India. Vol. V (H-K). Published by The Council of Scientific & Industrial Research, New Delhi; 1959; 155-157.
4. Ozturk N, Korkmaz S, Ozturk Y. Wound-healing activity of St. John's Wort (*Hypericum perforatum* L.) on chicken embryonic fibroblasts. J Ethnopharmacol 2007; 111(1):33-39.
5. Kumar V, Singh PN, Bhattacharya SK. Anti-inflammatory and analgesic activity of Indian *Hypericum perforatum* L. Indian J Exp Biol 2001; 39 (4):339-343.
6. Sosa S, Pace R, Bornancin A, et al. Topical anti-inflammatory activity of extracts and compounds from *Hypericum perforatum* L. J Pharm Pharmacol 2007; 59(5):703-709.
7. Schempp CM, Kirkin V, Simon-Haarhaus B, et al. Inhibition of tumour cell growth by hyperforin, a novel anticancer drug from St. John's wort that acts by induction of apoptosis. Oncogene 2002; 21:1242-1250.
8. Medina MA, Martinez-Poveda B, Amores-Sanchez MI, Quesada AR. Hyperforin: More than an antidepressant bioactive compound? Life Sci 2006; 79:105-111.
9. Chatterjee SS, Bhattacharya SK, Wonnemann M, Singer A, Muller WE. Hyperforin as a possible antidepressant component of Hypericum extracts. Life Sci 1998; 63(6):499-510.
10. Bhattacharya SK, Chakrabarti A, Chatterjee SS. Activity profiles of two hyperforin-containing Hypericum extracts in behavioural models. Pharmacopsychiatry 1998; 31: 22-29.

11. Kumar V. Singh PN, Jaiswal AK, Bhattacharya SK. Antidepressant activity of Indian *Hypericum perforatum* Linn in rodents. Indian J Exp Biol 1999; 37(12):1171-1176.
12. Kumar V, Singh PN, Muruganandam AV, Bhattacharya SK. Effect of Indian *Hypericum perforatum* Linn. on animal models of cognitive dysfunction. J Ethnopharmacol 2000; 72:119-128.
13. Deutscher Arzneimittel Codex (DAC) 91 edition, 3rd suppl. 1986.
14. Nahrstedt A, Butterweck V. Biologically active and other chemical constituents of the herb of *Hypericum perforatum* L. Pharmacopsychiatry1997; 30:129-134.
15. Vessal M, Hemmati M, Vasei M. Hypoglycemic effects of quercetin in streptozocin-induced diabetic rats. Comp Biochem Physiol C Toxicol Pharmacol 2003; 135C:357-364.
16. Coskun O, Kanter M, Korkmaz A, Oter S. Quercetin, a flavonoid antioxidant, prevents and protects streptozotocin induced oxidative stress and β-cell damage in rat pancreas. Pharmacol Res 2005; 51:117-23.
17. Menegazzi M, Novelli M, Beffy P, et al. Protective effects of St. John's wort extract and its component hyperforin against cytokine-induced cytotoxicity in a pancreatic β-cell line. Int J Biochem Cell Biol 2008; 40:1509-1521.
18. Sabu MC, Kuttan R. Anti-diabetic activity of medicinal plants and its relationship with their antioxidant property. J Ethnopharmacol 2002; 81:155-160.
19. Raut NA, Gaikwad, NJ. Antidiabetic activity of hydro-alcoholic extract of *Cypreus rotundus* in alloxan induced diabetes in rats. Fitoterapia 2006; 77:585-588.
20. Venkatesh S, Thilagavathi J, Shyam sunder D. Anti-diabetic activity of flowers of *Hibiscus rosasinensis*. Fitoterapia 2008; 79:79-81.
21. Lenzen S. The mechanisms of alloxan and streptozotocin-induced Diabetes. Diabetologia 2008; 51:216-226.