Original Article

Beneficial effects of a standardized *Hypericum perforatum* extract in rats with experimentally induced hyperglycemia

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ABSTRACT: The main aim of this study was to test the therapeutic potential of a standardized *Hypericum perforatum* extract in treating metabolic disturbances commonly associated with type-2 diabetes mellitus. Daily oral administration of the *Hypericum perforatum* extract (100, 200, and 300 mg/kg/day) for 14 consecutive days counteracted in a dose-dependent manner the alterations in blood glucose levels and lipid profile as well as liver glycogen content and body weight changes observed in a rat mode of nicotinamide-streptozotocin-induced diabetes. In general, effects of the highest dose of the extract in this model were quite similar, but not identical, to those of a 10 mg/kg/day dose of glibenclamide. The effects of single oral doses of the extract in a rat oral glucose tolerance test conducted in fasted animals were also analogous to those of an antidiabetic drug therapeutic use. These observations not only further expand the therapeutic potentials of Hypericum extracts but also indicate that stimulation of insulin release could be involved in their modes of actions. The importance of an extract with diverse, therapeutically interesting pharmacological properties is also briefly discussed.

Keywords: *Hypericum perforatum*, streptozotocin, nicotinamide, diabetes mellitus, NIDDM

1. Introduction

Different types of concoctions obtainable from the perennial herb *Hypericum perforatum* (also commonly called St. John's wort) have been known to have diverse medicinal uses for centuries (*1*). However, most reports dealing with the herb's clinical efficacy and bioactivities of its extracts over the past few decades have largely focused only on their antidepressant-like and other CNS-modulating effects (*2*,*3*). Information available on activity profiles of a hydroalcoholic extract of *Hypericum perforatum* (Hp) in animal models has revealed that such extracts could have other diverse therapeutic potentials and that hyperforin is likely to be a major bio-active constituent involved in their therapeutically interesting bio-activities (*4*). Although hyperforin is quantitatively the major secondary metabolite of the plant, several other therapeutically interesting bio-active phenolic components of the herb are also known. Since such components of the extract possess strong antioxidant properties (*5*-*7*), several reports based on this property of Hypericum extracts have also appeared in recent years. However, two facts that should be emphasized are that the bio-active constituents and activity profiles of diverse types of extracts vary considerably and that observations made with a specific type of extract may not be necessarily valid for other types as well.

Like in other parts of the globe, the diverse healing properties of *Hypericum perforatum* found on the Indian subcontinent have long been known (*8*,*9*). Unlike in the Western world, however, the herb was not traditionally considered to be a psychoactive plant in India. The very first report on antidepressant-like effects of an Indian Hypericum extract appeared only at the end of the past century (*10*). Although the extract tested in this study was prepared by a procedure analogous to those commonly used in the Western world to obtain hydroalcoholic Hypericum extracts, its analytically definable chemical constituents were unlike those widely in commercial use outside India. Unlike other Hypericum extracts, it was rich in xanthones (*11*,*12*). Like other extracts in commercial use, however, it also contained hyperforin and other bio-active phenolics and flavonoids.

In light of observations made with diverse types of Hypericum extracts, efforts are underway at the authors' laboratories to better pharmacologically and toxicologically characterize different types of Hypericum extracts and their known bio-active constituents. The ultimate goal of these efforts is to be able to design appropriate clinical studies for Hypericum extracts

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and/or their chemical constituents based on their broad preclinical activity profile. Therefore, earlier studies were conducted to define the activity profile of a standardized extract of *Hypericum perforatum* growing in India in a battery of animal models currently in wide use for drug discovery purposes or to define the activity profiles of known drugs (*13-19*).

The present paper describes the results of several experiments designed to test the effects of a Hypericum extract commonly in therapeutic use in the Western world (*i.e.,* standardized to containing at least 3% hyperforin and 0.3% hypericines) on metabolic disturbances often encountered in type-2 diabetic patients. In the first set of reported experiments, possible effects of repeated oral doses of the extract in the nicotinamide-streptozotocin rat model were tested. During the course of these experiments, a report (*20*) describing beneficial effects of hyperforin and Hypericum extract on insulin release came to the authors' attention. Since that study used *in vitro* models, testing whether the same would also hold true in intact animals was an interesting question. Consequently, the second set of reported experiments quantified the possible effect of single acute doses of the extract in a glucose tolerance test. Results of this experiment indicate that stimulation of insulin release could be involved in the observed effects of the extract in the type-2 diabetes model used.

2. Materials and Methods

2.1. *Animals*

Adult Charles-Foster rats (180 ± 10 g) were obtained from the Central Animal House of the Institute of Medical Sciences, Banaras Hindu University, Varanasi. The animals were housed in groups of six in polypropylene cages at an ambient temperature of 25 ± 1ºC and 45-55% relative humidity with a 12:12 h light/dark cycle. Except when otherwise mentioned, animals were always provided with commercial food pellets and water *ad libitum*. All animals were acclimatized to laboratory conditions for at least one week before their use in experiments, and each animal was subjected to experimental procedures only once. Principles of laboratory animal care (NIH publication number 85-23, revised 1985) guidelines were followed.

2.2. *Plant extract*

The tested hydro alcoholic extract of *Hypericum perforatum* (Hp) was obtained from Indian Herbs Research & Supply Co. Ltd., Saharanpur, UP, India. It was standardized (HPLC) to contain no less than 3.0% hyperforin and 0.3% hypericines. Thus, the tested extract can be considered to be a representative of Hypericum extracts commonly used in the Western world for therapeutic purposes as an antidepressant.

2.3. *Drug administration*

The extract was suspended in 0.3% carboxy methyl cellulose (CMC) and was orally administered through stainless steel oral gavage at three dose levels, *viz.* 100, 200, and 300 mg/kg of body weight. In hyperglycaemic rat experiments, daily single treatment continued for 14 consecutive days. Doses of the extract were chosen on the basis of earlier studies by this laboratory (*10,14,15*). Control rats received an equal volume of CMC, while glibenclamide (10 mg/kg body weight) was orally administered to another group of animals (positive control group). For an oral glucose tolerance test, the same doses of the extract as well as those of the standard drug were orally administered only once.

2.4. *Oral glucose tolerance test*

An oral glucose tolerance test was performed to evaluate the effects of the extract on peripheral glucose utilization in normal rats. Albino rats of either sex were divided into five groups (n = 6), fasted overnight, and orally administered 0.3% CMC, Hypericum extract (100, 200, and 300 mg/kg), or glibenclamide (10 mg/kg). Thirty minutes after the treatment, glucose (2 g/kg) was orally administered to all treatment groups. Blood glucose levels were quantified in blood samples collected by retro-orbital puncture prior to glucose administration as well as 30, 60, and 120 min thereafter.

2.5. *Induction of NIDDM*

A standardized procedure was used to induce hyperglycemia in rats (*21*). NIDDM was induced in animals fasted overnight by a single intraperitoneal injection of 65 mg/kg streptozotocin (Merck, Germany) 15 min after the *i.p.* administration of 120 mg/kg nicotinamide (SD fine Chem, India). Current pharmacology protocols (*22*) were followed for preparation and administration of a streptozotocin solution. Hyperglycemia was confirmed by blood glucose level determination conducted on the 3rd and 7th day after the streptozotocin injection. Rats with consistent hyperglycemia on the 7th day (fasting blood glucose levels > 250 mg/dL) were used to test the effects of the extract on type-2 diabetes.

Animals were divided into six groups of six rats each: Group I – Normal control rats treated with 0.3% CMC; Group II – Diabetic control rats treated with 0.3% CMC; Group III – Diabetic rats treated with Hp 100 mg/kg/day; Group IV – Diabetic rats treated with Hp 200 mg/kg/day; Group V – Diabetic rats treated with Hp 300 mg/kg/day; and Group VI – Diabetic rats treated with glibenclamide 10 mg/kg/day. Blood samples were collected by retro-orbital puncture and

the fasting blood glucose level was estimated on day 0 before the start of treatment, and on the 14th day of treatment, with a commercially available biochemical kit (Span Diagnostics Ltd., India). On the 14th day, plasma lipid profiles were also estimated using biochemical kits (Span Diagnostics Ltd., India). The liver glycogen content of rats was estimated using an anthrone reagent (*23*). Body weight changes of rats were periodically recorded throughout the experiment.

2.6. *Statistical analysis*

The mean ± standard error of the mean (SEM) was calculated for the values observed in each experimental group. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. GraphPad InStat (version 3.06) software was used for statistical analysis.

3. Results

3.1. *Glucose tolerance test*

Results of this experiment are summarized in Figure 1. Mean blood glucose levels of all groups before glucose loading were not statistically different from one another. After oral glucose loading, however, blood glucose levels in the group treated with the extract and the group treated with glibenclamide were significantly lower than those of the control group. Such was the case at every time point after glucose loading. These results reveal that like a standard anti-diabetic drug used in this experiment, the extract probably facilitates glucose utilization by stimulating glucose induced insulin release. Since blood insulin levels were not measured, such intervention must be experimentally verified in later studies. In any case, effects of the extract were dose-dependent, and the extract's maximum effects were observed within the first hour after an oral glucose challenge. Observed effects of a 200 mg/kg dose of the extract were more pronounced than those of lower doses. However, there was no statistically significant difference between the effects of 200 and 300 mg/kg doses of the extract. The maximum ceiling dose of the extract in this test is apparently 200 mg/kg and the effect of higher doses of the extract is qualitatively and quantitatively comparable to that of 10 mg/kg glibenclamide.

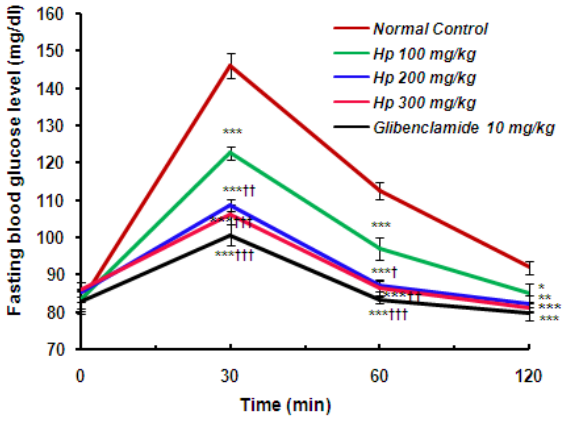

Figure 1. Effect of the Hp extract on an oral glucose tolerance test in normal rats. Values are mean ± SEM of 6 animals in each group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ *vs.* normal control; † $p < 0.05$, †† $p < 0.01$, ††† $p < 0.001$ *vs.* Hp 100 mg/kg.

3.2. *Blood glucose levels in an NIDDM model*

Compared to the normal control group, fasting blood glucose levels on day 0 of all groups subjected to a nicotinamide-streptozotocin challenge were significantly and equally higher (see Figure 2). In the diabetic control group, blood glucose levels remained elevated until day 14 of the experiment. However, diabetic animals receiving oral administration of an Hp extract (100, 200, and 300 mg/kg/day) for 14 consecutive days reduced fasting blood glucose levels in a dose-dependent manner. On day 14, there was no statistically significant difference between the blood glucose levels of the diabetic group treated with 300 mg/kg/day extract and the normal control group, and such was also the case for the diabetic group treated with 10 mg/kg/day glibenclamide. Analogous to findings of the glucose tolerance test, no statistically significant difference between the effects of 200 and 300 mg/kg/day doses of Hp extract was observed.

3.3. *Lipid profile of diabetic rats*

On the 14th day of the experiment, rats subjected to a nicotinamide-streptozotocin challenge (diabetic control

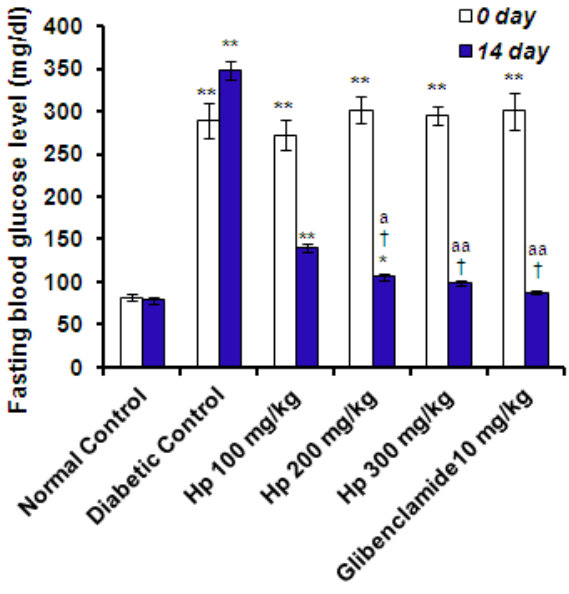

Figure 2. Effect of the Hp extract on the blood glucose level of rats with nicotinamide-streptozotocin-induced diabetes. Values are mean ± SEM for 6 animals in each group. * $p < 0.05$, ** $p < 0.001$ *vs.* normal control; † $p < 0.001$ *vs.* diabetic control; [a] $p < 0.01$, [aa] $p < 0.001$ *vs.* Hp 100 mg/kg.

Table 1. Effect of the Hp extract on lipid profile of rats with nicotinamide-streptozotocin-induced diabetes

Group (n = 6)	TC (mg/dL)	TG (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)
Normal control	79.53 ± 2.29	46.85 ± 1.47	38.67 ± 0.77	31.48 ± 2.59
Diabetic control	143.27 ± 6.68**	115.31 ± 6.58**	24.47 ± 1.43**	95.74 ± 7.1**
Hp 100 mg/kg	103.8 ± 1.78**†††	78.07 ± 3.24**†††	28.18 ± 0.40**†	60.00 ± 2.36**†††
Hp 200 mg/kg	85.67 ± 2.69$^{\dagger\dagger a}$	59.16 ± 1.7$^{\dagger\dagger aa}$	31.74 ± 0.3$^{**\dagger\dagger a}$	42.09 ± 3.2 $^{\dagger\dagger a}$
Hp 300 mg/kg	81.29 ± 3.26$^{\dagger\dagger aa}$	55.86 ± 3.22$^{\dagger\dagger aa}$	32.24 ± 0.76$^{**\dagger\dagger a}$	37.88 ± 3.94$^{\dagger\dagger aa}$
Glibenclamide 10 mg/kg	73.1 ± 2.55$^{\dagger\dagger aaa}$	69.37 ± 2.4*††	33.94 ± 0.77$^{\dagger\dagger aaa}$	25.28 ± 2.71$^{\dagger\dagger aaa}$

Values are mean ± SEM, n = number of animals in each group. $^{*}p < 0.01$, $^{**}p < 0.001$ *vs.* normal control, $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.001$ *vs.* diabetic control, $^{a}p < 0.05$, $^{aa}p < 0.01$, $^{aaa}p < 0.001$ *vs.* Hp 100 mg/kg.

group) showed a significant elevation in plasma total cholesterol (TC), triglycerides (TG), and low-density lipoprotein-cholesterol (LDL-C) levels while their plasma high-density lipoprotein-cholesterol (HDL-C) levels decreased significantly. Administration of an Hp extract or glibenclamide effectively restored these parameters to the physiological values observed in the normal control group (see Table 1). Here again, the effects of Hp 200 and 300 mg/kg/day doses of extract were comparable to those of a dose of glibenclamide (10 mg/kg/day).

3.4. *Liver glycogen of diabetic rats*

In comparison to the normal control group, a significant reduction in the liver glycogen content of the control diabetic group was observed. The results summarized in Figure 3 demonstrate that like glibenclamide, the extract reversed this effect of the nicotinamide-streptozotocin challenge and that its observed effect was dose-dependent. Quantitatively, the effect of Hp 300 mg/kg/day was again similar to that of 10 mg/kg/day glibenclamide.

3.5. *Body weight*

The body weight changes in control, diabetic control, and diabetic rats treated with the extract or with glibenclamide are summarized in Table 2. Animals in the normal control group gained about 4 g body weight over 14 days, whereas those in the diabetic control group lost about 24 g during this period. The mean loss in body weights of diabetic groups treated with Hp 100 and Hp 200 mg/kg/day were about 14 and 11 g, respectively, which was significantly less than that observed in the diabetic control group. In contrast, the diabetic group treated with Hp 300 mg/kg/day gained about 3 g body weight during the treatment period. The body weight loss of the diabetic group treated with glibenclamide (10 mg/kg/day) was significantly less than that of diabetic control group. Glibenclamide's effect on this parameter was comparable to that of Hp 200 mg/kg/day. Thus, a treatment regimen of Hp 300 mg/kg/day compensated for almost all of the quantified metabolic effects of a nicotinamide-streptozotocin challenge and also facilitated a return in body weight to normal levels.

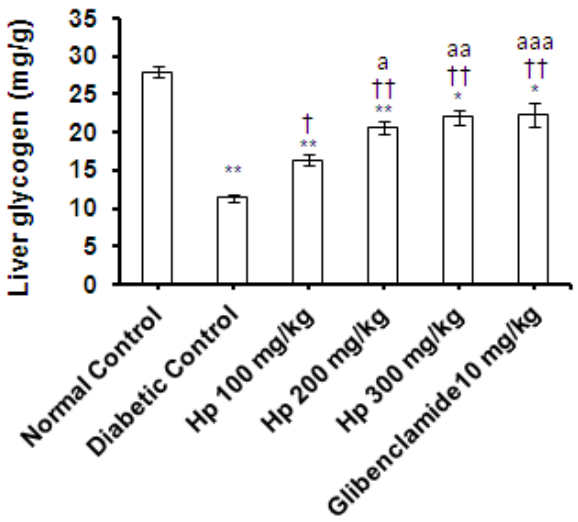

Figure 3. Effect of the Hp extract on liver glycogen content of rats with nicotinamide-streptozotocin-induced diabetes. Values are mean ± SEM for 6 animals in each group. $^{*}p < 0.01$, $^{**}p < 0.001$ *vs.* normal control; $^{\dagger}p < 0.01$, $^{\dagger\dagger}p < 0.001$ *vs.* diabetic control; $^{a}p < 0.05$, $^{aa}p < 0.01$, $^{aaa}p < 0.001$ *vs.* Hp 100 mg/kg.

Table 2. Effect of the Hp extract on body weight in rats with nicotinamide-streptozotocin-induced diabetes

Group (n = 6)	Body weight (g)	
	Initial (0 day)	Final (14th day)
Normal control	186 ± 2.19	190.67 ± 2.06
Diabetic control	183 ± 2.37	159 ± 3.52$^{*\$\$}$
Hp 100 mg/kg	184 ± 3.57	170.67 ± 3.5$^{*\dagger\$}$
Hp 200 mg/kg	188.17 ± 3.54	177 ± 4.09*†
Hp 300 mg/kg	182.54 ± 4.27	185.83 ± 2.69$^{\dagger ab}$
Glibenclamide 10 mg/kg	185.16 ± 3.8	176.5 ± 3.08*†

Values are mean ± SEM, n = number of animals in each group, $^{*}p < 0.001$ *vs.* normal control, $^{\dagger}p < 0.001$ *vs.* diabetic control, $^{a}p < 0.001$ *vs.* Hp 100 mg/kg, $^{b}p < 0.01$ *vs.* Hp 200 mg/kg, $^{\$\$}p < 0.001$, $^{\$}p < 0.05$ *vs.* day 0 for the same group.

4. Discussion

Taken together with the known pharmacological activity profile of Hypericum extracts in animal models, the observations of this study provide further experimental

evidence that the therapeutic potential of the herb is broader than that commonly expected based on existing knowledge of the medicinal uses of this plant. Indeed, findings revealed that the treatment regimen generally used in animal models to observe antidepressant-like and other CNS-modulating effects of Hypericum extracts is also effective in counteracting several metabolic disturbances encountered in type-2 diabetic animals. The effects of the extract on blood glucose levels and other metabolic parameters used in this study were dose-dependent. In general, the observed effects of a 300 mg/kg/day dose were almost identical to those of 10 mg/kg/day glibenclamide. However, the beneficial action of this dose of extract on the body weights of diabetic animals was more pronounced than that of a recognized anti-diabetic agent with potent ability to stimulate insulin release. Thus, the extract could have beneficial effects independent of its possible stimulation of insulin release.

In an oral glucose tolerance test conducted in normal rats after single oral doses of the Hp extract (100, 200, and 300 mg/kg) and glibenclamide, the effects of the extract were comparable to those of glibenclamide. These observations strongly suggest that like many therapeutically used anti-diabetic drugs (*24*), stimulation of insulin release could be involved in the effects of the extract observed in the type-2 diabetes model. A recent finding that hyperforin modulates insulin secretion under *in vitro* conditions (*20*) agrees with this assumption. However, the question whether only hyperforin is involved in the spectrum of effects observed with the Hp extract in a diabetes model or in an oral glucose tolerance test cannot be answered with certainty.

In line with the strategy being developed based on other medicinal plant-based drug discovery projects (*25*), previous studies have revealed diverse activity involving CNS modulation (*10,15-17*) by Hypericum extracts as well as their potential anti-inflammatory (*14*) and glibenclamide-like antidiabetic action (*18*). Initially, experiments examining the effect of a Hypericum extract in diabetes models were planned to test the possibility that their phenolic components with antioxidant and/or free radical scavenging properties could have beneficial effects on oxidative damage in the pancreas and other peripheral tissues. Taken together, the results presented in this and an earlier report (*18*) strongly suggest that such mechanisms may not be necessarily the only ones involved in its observed effects on metabolic disorders.

Research has established that hyperforin, one of the known bio-active components of the Hp extract used in this study, is a potent stimulator of diverse types of neurotransmitters under *in vitro* conditions (*26*). The effects of this extract component are not due to its direct interactions with specific neurotransmitter transporters and are probably due to its effects on cellular ionic homeostatic mechanisms (*27*). Since such mechanisms are also known to be involved in the control of glucose metabolism and insulin release (*28,29*), hyperforin may, by virtue of its modulation of cellular ionic concentration, also be involved in the observed effects of Hp extracts in diabetic animals.

Recent observations of the authors' laboratories (*30*) have revealed that, in a rat model of streptozotocin-induced diabetes, the effects of pure hyperforin (10 mg/kg/day; *i.p.*) are somewhat analogous to those of the Hypericum extract used in this study. Hyperforin has been reported to release acetylcholine in the rat brain (*31,32*). Acetylcholine may subsequently stimulate muscarinic M3 receptors in pancreatic cells (*33,34*) and augment insulin release, resulting in anti-hyperglycemic activity. Hyperforin has also been reported to activate cation channels of the transient receptor potential (TRP) family (*35*). Interestingly, the TRP channels of pancreatic β cells are also activated during glucose-stimulated insulin secretion (*29*). Thus, TRP channels could be a possible target of hyperforin in stimulating insulin secretion.

In conclusion, standardized extracts of Hp have a beneficial effect in a rat model of NIDDM and preliminary observations indicate that hyperforin may be at least partially responsible for the observed activity of Hp extracts though additional evidence will be provided by future studies.

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