TANGHUMANITAS MEDICINE

Original Article

Role of hyperforin in diabetes and its associated hyperlipidemia in rats

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ABSTRACT

The aim of the present study was to evaluate the possible roles of hyperforin against hyperglycemia, hyperlipidemia and oxidative stress in streptozotocin-induced diabetic rats. Diabetes was induced by a single intraperitoneal injection of streptozotocin (65 mg/kg). Biochemical parameters were measured following hyperforin treatment (10 mg/kg, i.p.) for 7 days. Hyperforin treatment significantly reversed the elevations in plasma glucose, triglycerides, total cholesterol and LDL-cholesterol. Hyperforin also reversed the declines in plasma HDL-cholesterol and liver glycogen, but did not reverse the change in plasma insulin levels when compared to the diabetic control rats. Hyperforin treatment also reversed the oxidative stress induced by streptozotocin. Moreover, the effect of the hyperforin on peripheral glucose utilization in normal rats was evaluated by an oral glucose tolerance test (OGTT). Hyperforin treatment significantly increased ($p < 0.05$) the glucose tolerance compared to the vehicle in OGTT. The antihyperglycemic, antihyperlipidemic and antioxidant activities of hyperforin (10 mg/kg, i.p.) were comparable qualitatively to glibenclamide (1 mg/kg, p.o.). In conclusion, we report for the first time through an *in vivo* study that hyperforin is potentially valuable for the treatment of diabetes and its associated hyperlipidemia and oxidative stress by enhancing the glucose utilization by peripheral tissues such as muscle and adipose tissues.

Keywords hyperforin, diabetes, insulin, antihyperglycemic, antihyperlipidemic, antioxidant

INTRODUCTION

Diabetes mellitus is a chronic illness which has been one of the major world health problems. It is a group of disorders characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins as well as an increased risk of complications from vascular disease (Scott et al., 1999; Stefan, 2005). It not only leads to hyperglycemia but also causes many complications, such as hyperlipidemia, hyperinsulinemia, hypertension, and atherosclerosis (Alberti et al., 1997).

Hyperforin is a polyprenylated acylphloroglucinol derivative of *Hypericum perforatum*, also known as St. Johns Wort (SJW) (Beerhues, 2006). The ethanolic extract of SJW contains 1-5% hyperforin (Lang et al., 2002). Hyperforin is one of the main constituents responsible for the antidepressant activity of SJW (Chatterjee et al., 1998a; Lakkmann et al., 1998). Apart from this antidepressant activity, hyperforin has also been shown to produce antibacterial, anti-inflammatory and antitumoral activities (Albert et al., 2002; Medina et al., 2006; Schwarz et al., 2003). Kumar et al. (2001) showed that a standardized extract of SJW containing hyperforin has significant anti-stress activity. SJW has also shown significant activity against stress-originated disorders such as mild to moderate depression (Chatterjee et al., 1998b; Kumar and Chatterjee, 2008; Kumar et al., 2003; Kumar et al., 1999), aggression (Husain et al., 2009a) and cognitive dysfunction (Kumar et al., 2002; Kumar et al., 2000). It is reported that stress could also cause endocrine related disorders, especially diabetes, by causing the activation of the hypothalamic-pituitary-adrenal (HPA) axis (Radahmadi et al., 2006). As mentioned above, since SJW shows significant activity against stress and stress related disorders, SJW and its component hyperforin could also possibly be active against diabetes, a stress related disorder. The role of oxidative stress in diabetes mellitus cannot be ruled out (Baynes, 1991; Rosen et al., 2001; Giugliano et al., 1996). There are few reports explaining the efficacy of SJW extract as an antioxidant (Dubey et al., 1999; El-Sherbiny, 2003; Sanchez-Reus et al., 2007; Silva et al., 2008). Hyperforin has also been shown to possess antioxidants (Feisst and Werz, 2004; Heilmann, 2003).

In a recent *in vitro* study, hyperforin and SJW extract showed a protective effect against cytokine-induced pancreatic β cell damage in a INS-1E β-cell line (Menegazzi et al., 2008). Our recent studies with 50% ethanolic extract (standardized to contain hyperforin, NLT 3%, by HPLC) of SJW have shown significant antidiabetic activity in alloxan- and streptozotocin-induced diabetic rats (Husain et al., 2008, Husain et al., 2009b). Furthermore, another study reveals the antihyperlipidemic activity of hyperforin in fructose-fed hyperlipidemic rats (Ineedi and Kumar, 2009). Keeping these facts in mind, the aim of the present study was to investigate the role of hyperforin in the observed antidiabetic, antihyperlipidemic and antioxidant activities of SJW extract in diabetic rats.

MATERIALS AND METHODS

Animals and drugs
Charles Foster albino rats of either sex weighing 150 ± 20 g were obtained from the Central Animal House of Institute of Medical Sciences, Banaras Hindu University, Varanasi. Temperature (25 ± 5℃) and humidity (55 ± 10%) were maintained constant and a 12 hr light and 12 hr dark cycle was followed. Animals were habituated to the environment for 7 days before performing the experiment and were allowed to access food and water *ad libitum*. The experimental procedures were in compliance with National Institute of Health (NIH) Guide for Care and Use of Laboratory Animals (NIH Publication No. 80-23; revised 1978).

Hyperforin was a gift sample from Dr. Willmar Schwabe GmbH & Co. KG (Karlsruhe, Germany). Streptozotocin was purchased from Calbiochem (Darmastadt, Germany) and Glibenclamide was a gift sample from Cipla (Vikhroli, India).

Antidiabetic and antihyperlipidemic effects
Animals were divided into four groups (6 animals each) as Group I (non-diabetic control), Group II (diabetic control), Group III (hyperforin-treated diabetic) and Group IV (glibenclamide-treated diabetic).

To induce diabetes, rats of group II-IV were injected with freshly prepared streptozotocin (65 mg/kg, i.p.) in 0.01 M sodium citrate buffer of pH 4.5. Group I rats were injected with sodium citrate buffer. Seven days after the administration of streptozotocin, plasma glucose levels were determined. Animals were considered diabetic if they showed a blood glucose level of at least 190 mg/dl (Akah et al., 2009) and only those rats were included in the study. Drug treatment was started seven days after the streptozotocin administration.

Group I and Group II rats were treated orally with the vehicle. Group III and Group IV rats were treated with hyperforin (10 mg/kg/day for 7 days, i.p.) and gibenclamide (1 mg/kg/day for 7 days, p.o.) respectively. Hyperforin was

suspended in 1% dimethylsulfoxide that contained 0.3% carboxymethylcellulose. On the 7th day, after 1 hr of administration of last dose, blood samples were collected from overnight fasted rats. Plasma glucose, triglyceride and cholesterol levels were determined by spectrophotometric methods using commercially available assay kits. Plasma insulin was estimated by an enzyme-linked immunosorbent assay (ELISA kit; DRG Diagnostics, GmbH, Germany). Liver glycogen was determined by an anthrone reagent method (Sharma et al., 2008; Vies, 1953).

For checking anti-oxidant status, rats were euthanized to collect the liver and pancreas. The liver and pancreas were washed in ice-cold saline to remove the blood. Thereafter, the liver and pancreas were homogenized in an ice-cold Tris-HCL buffer (0.025 M, pH 7.4) to yield a 10% (w/v) homogenate. The homogenate was centrifuged at 15000 X g for 30 min at 4^0C. The supernatant obtained was used for estimation of lipids per oxides (LPO), super oxide dismutase (SOD) and catalase (CAT). Protein levels were estimated using the method of Lowry et al., (1951). Lipid peroxidation was quantified by measuring the level of malonaldehyde (MDA) according to the method of Ohkawa et al., 1979. SOD activity was estimated by the method of Kakkar et al., 1984. Catalase activity was assayed by the method of Sinha (1972).

Furthermore, to evaluate the peripheral glucose utilization, an oral glucose tolerance test (OGTT) was performed on normal rats. Animals of either sex were divided into three groups (n = 6), and they were treated either with the vehicle or with hyperforin or with glibenclamide. Glucose (2 g/kg) was administered orally 30 min after the hyperforin and glibenclamide treatments. Blood glucose levels were determined in blood samples collected at 0 min (prior to glucose administration), 30, 60 and 120 min after glucose administration (Husain et al., 2011).

Statistical analyses
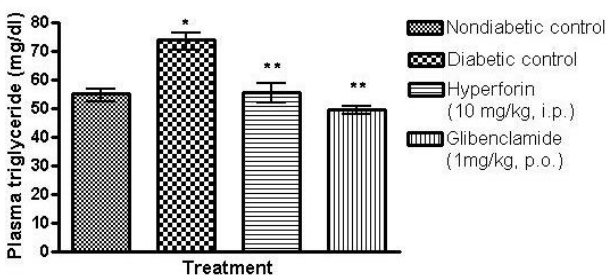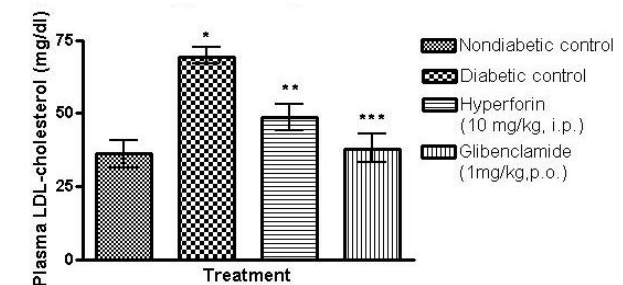Data are expressed as mean ± standard error of mean (SEM).
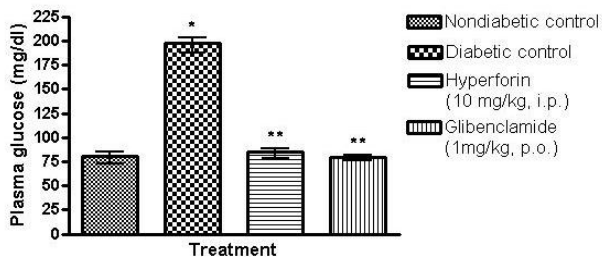
Fig. 1. Effect of hyperforin on plasma glucose level of rats. Values are given as mean ± SEM (n = 6). * and ** indicate *p* < 0.001, compare to nondiabetic control and diabetic control respectively.
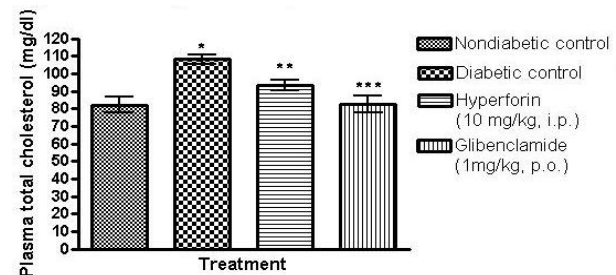
Fig. 2. Effect of hyperforin on plasma total cholesterol level of rats. Values are given as mean ± SEM (n = 6). * and *** indicate *p* < 0.001, compare to nondiabetic control and diabetic control respectively. ** indicates *p* < 0.05, compare to diabetic control

Fig. 3. Effect of hyperforin on plasma triglyceride level of rats. Values are given as mean ± SEM (n = 6). * and ** indicate *p* < 0.001, compare to nondiabetic control and diabetic control respectively.

Fig. 4. Effect of hyperforin on plasma total LDL-cholesterol level of rats. Values are given as mean ± SEM (n = 6). * and *** indicate *p* < 0.001, compare to nondiabetic control and diabetic control respectively. ** indicates *p* < 0.01, compare to diabetic control.

Table 1. Effect of hyperforin on oxidative status in liver and pancreas of rats

Groups	Liver			Pancreas		
	LPO	SOD	CAT	LPO	SOD	CAT
Nondiabetic control	6.36 ± 0.15	14.14 ± 0.21	22.78 ± 0.24	3.65 ± 0.12	3.61 ± 0.06	12.63 ± 0.22
Diabetic control	10.70 ± 0.17[*]	8.53 ± 0.17[*]	11.90 ± 0.21[*]	10.83 ± 0.19[*]	1.99 ± 0.05[*]	6.48 ± 0.17[*]
Hyperforin (10 mg.kg, i.p.)	8.01 ± 0.18[**]	11.55 ± 0.23[**]	17.93 ± 0.16[**]	6.85 ± 0.14[**]	2.82 ± 0.09[**]	9.36 ± 0.18[**]
Glibenclamide (1 mg/kg, p.o.)	7.34 ± 0.13[**]	12.90 ± 0.16[**]	20.56 ± 0.26[**]	5.32 ± 0.19[**]	3.20 ± 0.05[**]	11.08 ± 0.10[**]

Values are given as mean ± SEM (n = 6). LPO=Lipid peroxides, SOD= superoxide dismutase, CAT= Catalase. [*] and [**] indicate $p < 0.001$, compared to nondiabetic control and diabetic control respectively.

Statistical analyses were performed by one way analysis of variance (ANOVA) followed by a Student-Neuman Keuls multiple comparison test (Graphpad prism version 5).

RESULTS

Streptozotocin administration (65 mg/kg, i.p.) significantly increased fasting plasma glucose, total cholesterol, triglyceride and LDL-cholesterol levels, while levels of HDL-cholesterol, liver glycogen and plasma insulin were significantly decreased, compared with the control rats. Hyperforin (10 mg/kg, i.p.) and glibenclamide (1 mg/kg, p.o.) treatments significantly decreased the elevated plasma glucose, total cholesterol, triglyceride and LDL-cholesterol levels (Figs. 1-4 respectively), whereas HDL-cholesterol and liver glycogen levels were significantly increased (Figs. 5-6 respectively) compared with the diabetic control rats. The levels of insulin did not changed significantly in the hyperforin-treated rats when compared to the diabetic control rats (Fig. 7). Otherwise, the hyperforin- or glibenclamide-treated rats were comparable qualitatively to the non-diabetic control rats. There were significant increases in the levels of lipid peroxidation both in the liver and pancreas of diabetic control rats in comparison to the non-diabetic control

rats. At the same time, the levels of SOD and CAT decreased significantly in the liver and pancreas of the diabetic control rats in comparison to the non-diabetic control rats. However, hyperforin and glibenclamide significantly reversed these changes. The results are summarized in Table 1.

In OGTT, hyperforin significantly increased the glucose tolerance in comparison to vehicle-treated rats. The mean blood glucose levels of all groups before glucose administration were normal. Whereas after the glucose challenge, the blood glucose levels in the rats treated with hyperforin and glibenclamide were significantly lower than those of the control group at 30, 60 and 120 min. The results are depicted in Fig. 8.

DISCUSSION

Single intraperitoneal administration of streptozotocin at a dose of 65 mg/kg is used to induce diabetes in rats (Cetto et al., 2000). This single dose of streptozotocin causes the destruction of pancreatic β cells by alkylating DNA at O^6 position in guanine and also by liberating toxic amounts of nitric oxide which cause oxidative stress (Szkudelski, 2001). Production of nitric oxide depends on the expression of inducible nitric oxide synthase (iNOS), which in turn is regulated by two
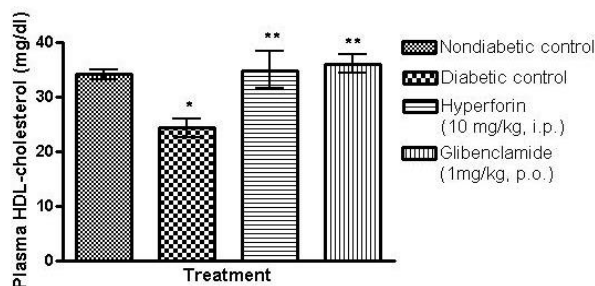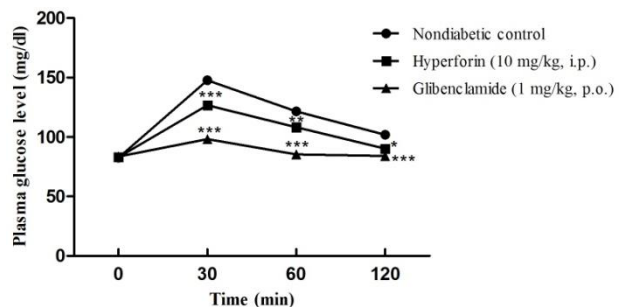
Fig. 5. Effect of hyperforin on plasma total HDL-cholesterol level of rats. Values are given as mean ± SEM (n = 6). [*] and [**] indicate $p < 0.01$, compare to nondiabetic control and diabetic control respectively.
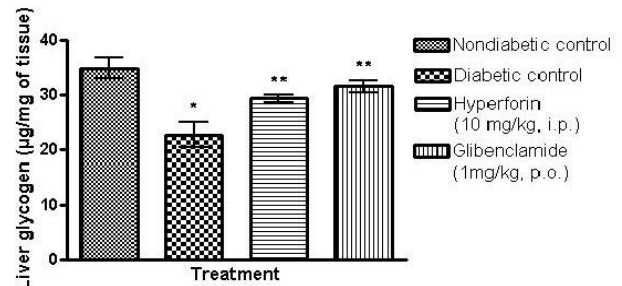
Fig. 6. Effect of hyperforin on liver glycogen level of rats. Values are given as mean ± SEM (n = 6). [*] and [***] indicate $p < 0.001$, compare to nondiabetic control and diabetic control respectively. [**] indicates $p < 0.01$, compare to diabetic control.
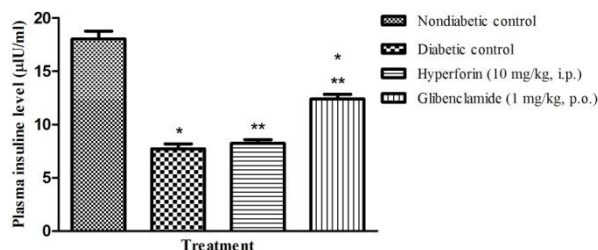
Fig. 7. Effect of hyperforin on plasma insulin level of rats. Values are given as mean ± SEM (n = 6). [*] and [**] indicate $p < 0.001$, compare to nondiabetic control and diabetic control respectively.

Fig. 8. Effect of hyperforin in oral glucose tolerance test on normal rats. Values are given as mean ± SEM (n = 6). [*], [**] and [***] indicate $p < 0.05$, $p < 0.01$ and $p < 0.001$, compare to nondiabetic control respectively.

transcription factors namely the nuclear factor kappaB (NF-kB) and the signal tranducer and activator of transcription-1 (STAT-1) (Ganster et al., 2001). Activation of these transcription factors is controlled by cytokines viz. IFN-γ, TNF-α and IL-1β (Mandrup-Poulson, 2003). In type 2 diabetes, increased cytokine production by adipose tissue because of metabolic derangement is the main cause of the destruction of pancreatic β cells and hence the decreased insulin secretion (Mandrup-Poulson, 2003). In an *in vitro* study, SJW extract and its component hyperforin were shown to down-regulate the cytokine-induced activations of both transcription factors NF-kB and STAT-1 in the INS-1E β cell line (Menegazzi et al., 2008). In the same study, SJW extract and hyperforin also hindered iNOS expression, offering protection against nitric oxide-induced oxidative stress.

Stress is one of the important causative factors of diabetes (Radahmadi, 2006; Strachan et al., 1997). Stress causes parallel activation of the HPA axis and sympatho-adrenomedullary system (Chrousos and Gold, 1992). The consequence of HPA axis activation is an increased release of glucocorticoids by the adrenal cortex, which activates all the processes that cause an increase in the blood glucose. Glucocorticoids also cause the insulin resistance of the target cells by altering gene expression (Chrousos and Gold, 1992). Development of insulin resistance alters blood glucose, the blood lipid profile and glycogen storage by the liver, which thus leads to the development of diabetes.

Diabetes itself stimulates the stress response. Increased HPA activity has been reported in patients suffering from diabetes (Roy et al., 1990). Hyperactivation of the HPA axis occurs in streptozotocin-treated rats also (Chan et al., 2002). The mechanism behind the increased activation of the HPA axis in humans and diabetic rats is not entirely clear. However, Chan et al. have proposed that the hyperactivation of the HPA axis in diabetes can be due to the decreased sensitivity of the glucocorticoid negative-feedback mechanism (Chan et al., 2002). Depression has a negative physiologic effect on glucose metabolism (Katon, 2008) and it is associated with an increased risk for developing diabetes (Lustman and Clouse, 2005). In recent studies, it has been shown that antidepressant drugs with serotonin reuptake-inhibiting activities, are associated with hypoglycemia (Derijks et al., 2008; Musselman et al., 2003) and hyperforin exerts its antidepressant effect by dint of its potent serotonin reuptake inhibitor activity (Chatterjee et al., 1998a; Singer et al., 1999).

Keeping in mind the aforementioned activities, i.e. the inhibition of cytokine-induced pancreatic β cell damage, anti oxidant and antidepressant (serotonin reuptake inhibition) activities, hyperforin could potentially be valuable in treating diabetes mellitus. However, to the best of our knowledge, no *in vivo* study has been reported so far supporting the antidiabetic activity of hyperforin. Unlike glibenclamide, hyperforin does not normalize the insulin levels in diabetic rats suggesting that hyperforin may exert an antihyperglycemic effect via a different mechanism than glibenclamide. However, in our study, elevated blood glucose levels by streptozotocin in diabetic rats were brought to normal by hyperforin (10 mg/kg, i.p.) treatment. Altered lipid profiles and decreased liver glycogen levels were also normalized by the hyperforin treatment indicating increased insulin sensitivity.

Insulin resistance plays an important role not only in the development and progression of diabetes mellitus but also in the establishment of the metabolic syndrome (Rader, 2007). These co-morbid metabolic disorders increase the risk of cardiovascular disorders. Type 2 diabetic patients have characteristic dyslipidemia with an overproduction of VLDL and hypertrigyceridemia, and the treatment of dyslipidemia in such patients has been successful in reducing cardiovascular disease (Ginsberg et al., 2005). However, due to limitations of available treatments for hyperlipidemia in Type 2 diabetes (Duntas and Kolovou, 2011) novel pharmacological targets and drug leads are now being sought for by many drug discoverers. Observations reported in this article, taken together with the ones reported earlier (Ineedi and Kumar, 2009), encourage us and suggest that hyperforin could be a lead molecule for identifying novel pharmacological targets and drug leads urgently needed for such purposes. In any case, they add further experimental evidence to our conviction that the metabolic effects of hyperforin are due to its extra-pancreatic effects.

The involvement of oxidative stress mechanisms has often been implicated in diabetes. Hyperforin, which is a polyprenylated acylphloroglucinol, has earlier been reported to possess antioxidant activities (Gioti et al., 2009). However, this conclusion was reached by observations made *in vitro*. In our study, hyperforin was found to exert significant antioxidant activity. It decreased LPO levels and increased the activities of SOD and CAT significantly in the liver and pancreas of diabetic animals, and analogous was also the case for the standard anti-diabetic drug used in this study. Thus, it seems reasonable to assume that the modulation of endogenous oxidative mechanisms and processes are involved in the observed beneficial effects of the agents on glucose homeostasis in diabetic animals.

The observations that hyperforin significantly suppressed blood glucose levels in the OGTT test conducted in diabetic rats indicate its ability to improve glucose consumption in peripheral tissues. However, quantitatively, the observed effect of hyperforin was much lower than that of glibenclamide (see Fig. 8). These observations indicate again that extra-pancreatic mechanisms are most probably involved in its anti-hyperglycemic activities.

Observations reported in this article strongly reveal that hyperforin is an antidiabetic secondary metabolite of SJW, and that antioxidative mechanisms are involved in its modes of action. Hyperforin has long been known to be the quantitatively major bioactive component of therapeutically-used SJW extracts, and during recent years numerous proposals on its mode of actions have been made. However, most such mechanistic reports deal with *in vitro* observations. Moreover, to-date, little attention has been paid to its antidiabetic properties. Our *in vivo* observations strongly suggest that protection of the pathological oxidative processes could be involved in its beneficial effects against metabolic disorder, and that it could be a structurally and functionally novel lead for combating the diabetic epidemic of the 21st century.

ACKNOWLEDGEMENTS

CONFLICT OF INTEREST

The authors do not have any conflict of interest in the present study.

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