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BENEFICIAL EFFECT OF *HYPERICUM PERFORATUM* ON DEPRESSION AND ANXIETY IN A TYPE 2 DIABETIC RAT MODEL

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Abstract: Recent studies have revealed diverse therapeutically interesting pharmacological properties of a standardized *Hypericum perforatum* extract (HpE) potentially useful for treatments of patients with metabolic and psychiatric disorders. Consequently, the presented experiments were designed to test usefulness of the extract for the treatment of comorbid conditions of mood disturbances and anxiety in diabetic rats. Type 2 diabetes mellitus was induced in overnight fasted rats by a single *i.p.* injection of streptozotocin (STZ; 65 mg/kg), 15 min after an *i.p.* injection of nicotinamide (120 mg/kg). HpE was administered orally (100 and 200 mg/kg b.w..) to diabetic animals for 14 days. Anxiolytic activity was evaluated using open-field exploration test (OFT) and elevated plus maze (EPM) test. Antidepressant activity was assessed using Porsolt's forced swim test (FST). Fasting blood glucose levels in different groups were analyzed on the 14th day. Diabetic rats showed significant increase in anxiety in OFT and EPM compared to non diabetic normal control rats. Diabetic rats treated with HpE have shown significant anxiolytic activity in OFT and EPM test. In FST, immobility period of vehicle treated diabetic rats was significantly increased ($p < 0.05$) compared to normal control rats. Treatment with HpE significantly decreased ($p < 0.001$) immobility period compared to vehicle treated diabetic control rats. HpE treatment significantly reduced elevated blood glucose levels in diabetic rats. The presented observations strongly suggest that HpE could be suitable alternative therapeutic option for prevention, as well as treatment, of comorbidities caused by, or associated with, depression, anxiety and diabetes.

Keywords: anxiety, depression, diabetes, *Hypericum perforatum*

Diverse medicinal uses of the perennial herb *Hypericum perforatum* L. (Clusiaceae) have been known since ages. During the past few decades numerous clinical reports and studies have demonstrated beneficial effects of its hydro-alcoholic extracts for helping patients with mild to moderately severe depressive symptoms. Critical analysis of available information on bioactivities and clinical efficacy of such extracts revealed that their antidepressant like efficacy and modes of actions cannot be like those of conventionally known synthetic antidepressant, anxiolytic or any other psychoactive drug known to date (1). Although hyperforin was initially identified as quantitatively the major antidepressant component of such extracts, currently available information on its therapeutically interesting bioactivity profile indicates that it could be a pharmacologically interesting molecule potentially useful for treatments of diverse types of disorders other than depression (2). In addition, it cannot be ignored that therapeutically used *Hypericum perforatum* extracts contain numerous other bioactive components with diverse spectrums of pharmacological activity profiles.

Our earlier studies have shown the efficacy of HpE in variety of CNS disorders including anxiety and depression in non-diabetic rodents (3–5). HpE showed beneficial effect in nicotinamide-streptozotocin induced diabetic rats and also inhibited rise in blood glucose level without causing overt hypoglycemia in oral glucose tolerance test in non-diabetic rats (6). In view of the observed broad spectrum of activity profile of Hypericum extracts in animal models, it seems to be a particularly good candidate for treatment of comorbid disorders involving disturbances of CNS functions and diabetes. Experiments described in this communication were therefore conducted to verify this possibility and to

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check whether antidepressant and anxiolytic effects of the extract is maintained in diabetic animals or not. Choices of the experimental models, treatment regimen and doses of the extract were selected on the basis of earlier studies.

MATERIALS AND METHODS

Animals

Adult Charles Foster rats (180 ± 10 g) were obtained from Central Animal House of 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 ± 1°C and 45–55% relative humidity, with a 12:12 h light/dark cycle. Animals were provided with commercial food pellets and water *ad libitum*, except stated otherwise. All the animals were acclimatized to laboratory conditions for at least one week before using them for the experiments. Principles of laboratory animal care guidelines (NIH publication number 85-23, revised 1985) were followed. Prior, approval from the Institutional Animal Ethics Committee was obtained.

Plant extract

The tested hydro-alcoholic extract of *Hypericum perforatum* (Clusiaceae) was obtained from Indian Herbs Research & Supply Co. Ltd., Saharanpur, UP, India. It was standardized (HPLC) to contain not less than 3.00% hyperforin and 0.3% hypericines. Thus, the tested extract can be considered to be a representative of Hypericum extracts commonly commercialized in the western world for therapeutic purposes as antidepressant.

Induction of diabetes

Non insulin-dependent diabetes was induced in overnight fasted rats (6, 7). Briefly, rats were given a single *i.p.* injection of streptozotocin (65 mg/kg; Merck, Germany), 15 min after *i.p.* administration of 120 mg/kg of nicotinamide (SD Fine Chem, India) (8). Hyperglycemia was confirmed by blood glucose analysis on the 3rd and 7th day after the streptozotocin injection. Rats with consistent hyperglycemia on 7th day (fasting blood glucose levels > 200 mg/dL) were considered diabetic and were used for further studies. Rats were divided into four groups (n = 6 rats per group) Group I – normal control rats (non-diabetic) treated with 0.3% carboxymethyl cellulose (CMC); Group II – diabetic control rats treated with 0.3% CMC; Group III and Group IV – diabetic rats treated with HpE 100 and 200 mg/kg/day, respectively, for 14 days.

Behavioral tests

Open-field exploration (OFT) and elevated plus maze (EPM) tests were used to evaluate anxiolytic activity, whereas antidepressant activity was assessed using Porsolt's forced swim test (FST). All the behavioral tests were performed on 14th day, 1 h after the last oral drug administration.

Open-field exploration test

Open-field exploratory behavior was quantified according to the procedure reported elsewhere (4). In short, each animal was centrally placed in the open-field apparatus for 5 min, during which the parameters quantified were: ambulation, rearing, self grooming, activity in centre and fecal droppings.

Elevated plus-maze test

Each rat was placed in the centre of the plus maze facing an open arm. During the 5 minutes test the number of entries into open and closed arm and the time spent in each arm were scored. A rat was taken to have entered an arm when its four legs were on the arm. In addition to the total number of arm entries, the number of entries into the open arms expressed as a percentage of total arm entries and the time spent on the open arms expressed as a percentage of the time spent on both the open and closed arms were assessed (4, 5). These two parameters were used as indices of anxiety.

Porsolt's forced swim test

Antidepressant activity was assessed using Porsolt's forced swim test (FST). The period of immobility (floating in water without struggling and making only those movements necessary to keep its head above water) during 5 min test period was recorded (3).

Estimation of blood glucose

Fasting blood glucose level was estimated by colorimetric assay based on glucose oxidase-peroxidase method using commercially available biochemical kit (Span Diagnostics Ltd., India).

Statistical analysis

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

Table 1. Effect of HpE on open field exploration test in rats.

Treatment	Ambulation (N)	Rearing (N)	Self Grooming (N)	Activity in center (N)	Fecal dropping (N)
Normal control (CMC)	42.67 ± 2.16	13.17 ± 1.01	24.83 ± 1.32	4.83 ± 0.44	3.50 ± 0.20
Diabetic control (CMC)	38.50 ± 2.45	8.83 ± 0.95*	18.17 ± 0.86**	3.00 ± 0.53*	3.83 ± 0.30
Diabetic + HpE 100 mg/kg	40.33 ± 2.11	11.5 ± 1.15	20.83 ± 0.86	5.17 ± 0.60$^{\$}$	3.17 ± 0.28
Diabetic + HpE 200 mg/kg	45.17 ± 2.27	13.5 ± 1.23$^{\$}$	21.67 ± 1.69	5.83 ± 0.55$^{\$\$}$	3.00 ± 0.33

*$p < 0.05$, $^{**}p < 0.01$, *vs*. normal control; $^{\$}p < 0.05$, $^{\$\$}p < 0.01$ *vs*. diabetic control (one-way ANOVA following by Student-Newman-Keuls test)

Table 2. Effect of HpE in elevated plus maze test in rats.

Treatment	Open arm entries (N)	Closed arm entries (N)	Time spent in open arm (s)
Normal control (CMC)	5.17 + 1.47	15.83 + 1.94	32.5 + 6.09
Diabetic control (CMC)	3.33 + 1.03*	18.5 + 3.33	20.00 + 3.58*
Diabetic + HpE 100 mg/kg	6.83 + 1.47$^{*\,\$\$}$	14.67 + 1.75$^{\$}$	44.83 + 12.92$^{*\,\$\$}$
Diabetic + HpE 200 mg/kg	8.67 + 1.37$^{***\,\$\$}$	14.00 + 1.41$^{\$}$	54.67 + 13.34$^{**\,\$\$}$

$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, *vs*. normal control; $^{\$}p < 0.05$, $^{\$\$}p < 0.001$, *vs*. diabetic control (one-way ANOVA following by Student-Newman-Keuls test)

RESULTS

Open-field exploration test

Vehicle treated diabetic control rats showed significant decrease ($p < 0.05$) in rearing and self grooming compared to normal control rats. Diabetic rats treated with HpE (100 and 200 mg/kg) showed significant ($p < 0.05$) increase in rearing and self grooming compared to vehicle treated diabetic control rats. The number of squares crossed (ambulation) did not change significantly in rats of different treatment groups. Activity in centre was also increased significantly in HpE treated group compared to vehicle treated diabetic control group. However, there was no significant difference in number of fecal droppings in different treatment groups in OFT (Table 1).

Elevated plus-maze test

Diabetic control rats show significant decrease ($p<0.05$) in number of open arms entries and the time spent in the open arms ($p<0.05$) compared to normal control group. Treatment with HpE (100 and 200 mg/kg) significantly increased the number of open arms entries and the time spent in the open arms (Table 2). Percent open arm entries and percent time spent in open arm are depicted in Figure 1a & 1b, respectively.

Porsolt's forced swim test

Diabetic rats showed increased immobility period ($p < 0.05$) compared to normal control rats. HpE treatment dose dependently decreased immobility period ($p < 0.05$ and $p < 0.001$ for HpE 100 and 200 mg/kg, respectively) compared to normal control rats (Fig. 2).

Blood glucose level

Fasting blood glucose level of different treatment groups are given in Table 3. HpE treatment significantly reduced ($p < 0.001$) fasting blood glucose level compared to vehicle treated diabetic rats.

DISCUSSION

Extensive efforts for the search of novel pharmacotherapies using traditionally known herbal remedies and modern medicinal chemistry are now being made in many laboratories. However, most such efforts concentrate only on some symptoms of the complex health problems, and continue to use target oriented reductionist principles of modern

Table 3. Effect of HpE treatment on fasting blood glucose level in rats.

Treatment	Blood glucose level (mg/dL)	
	0 day	14th day
Normal control (CMC)	79.34 + 3.17	81.76 + 3.51
Diabetic control (CMC)	284.99 + 12.04***	305.32 + 9.92***
Diabetic + HpE 100 mg/kg	286.09 + 6.82***	151.28 + 8.72***$
Diabetic + HpE 200 mg/kg	298.91 + 15.14***	119.5 + 4.89**$

** $p < 0.01$, *** $p < 0.001$, *vs*. normal control; $ $p < 0.001$, *vs*. diabetic control (one-way ANOVA following by Student-Newman-Keuls test)

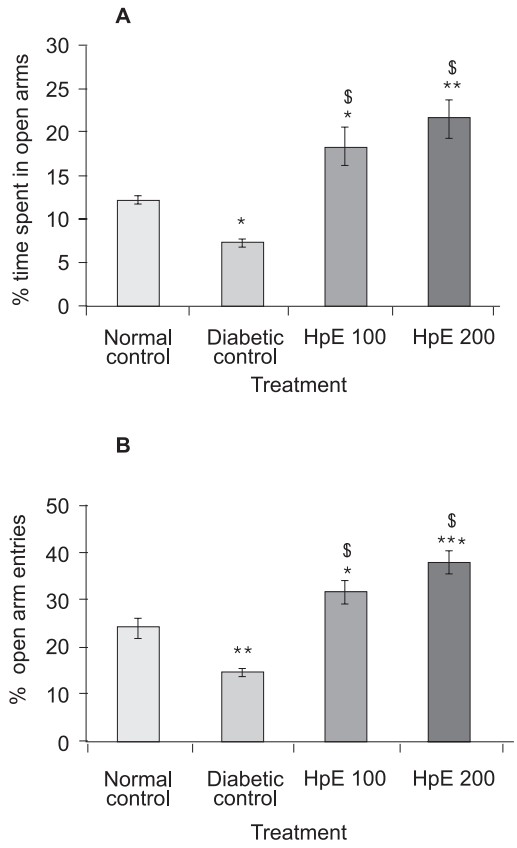

Figure 1. (**A**) Effect of HpE on percentage of open arm entries in elevated plus maze test in rats. (**B**) Effect of HpE on percentage of time spent in open arm in elevated plus maze test in rats. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ *vs*. normal control; $ $p < 0.001$ vs. diabetic control (one-way ANOVA followed by Student-Newman-Keuls test)

drug discovery. Thus, for example, although antidepressant and anxiolytic like efficacy of hyperforin and *Hypericum perforatum* have been known since long time, little efforts have been made to test their special therapeutic potential for helping diabetic patients with depression as comorbidity.

The experiments described in this communication were conducted as a part of ongoing project directed towards bioassay guided standardization of herbal remedies widely used in India by the practitioners of its endogenous systems of medicine. Since traditionally known uses of herbal preparations are in general based on the epistemology of knowledge supplied by ancient Indian medical systems, therefore, attempts are also made to experimentally verify, or rediscover, therapeutic possibilities offered by traditionally known medicinal herbs. However, the ultimate goal of this project is to identify affordable and therapeutically promising herbal extracts, which could be further developed for appropriate clinical trials, necessary for ascertaining therapeutic validity of preclinical observations. Amongst a few herbal extracts studied to date under this project, HpE is the most promising and extensively studied one.

The reported observations reveal that diabetic animals show increased anxiety and depression compared to non diabetic rats and that the antidepressant and anxiolytic effects of HpE are retained in diabetic animals as well. On the contrary, available literature clearly indicates that such is not the case for commonly clinically used antidepressants and anxiolytics (9–13). Although further comparative and other studies are necessary to clarify the situation, it remains certain that pharmacological classification of HpE as an antidepressant, or as a synaptosomal reuptake inhibitor only is no longer very justifiable.

It is now well established (14–16) that psychological risk factors, such as depression and anxiety are independently associated with an increased risk of different forms of diabetes and associated mortalities and other socio-economic problems (17). A meta-analysis reported that 11% of patients with diabetes met the criteria for comorbid major depressive disorder and 31% experienced significant depressive symptoms (18). Although depressive symptoms, anxiety and numerous cognitive dys-

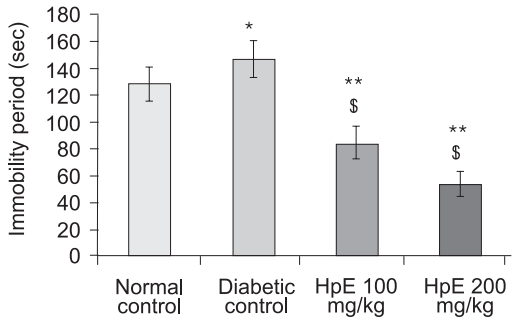

Figure 2. Effect of HpE in forced swim test in rats. * $p < 0.05$, ** $p < 0.001$, *vs.* normal control; $ $p < 0.001$, *vs.* diabetic control (one-way ANOVA followed by Student-Newman-Keuls test)

functions are now well established comorbidities in diabetic (14) patients, until now more attention has been paid to depression only. This might be due to the fact that available evidences from extensive population studies suggested that the relationship between diabetes and depression is bi-directional (19) and that this is not necessarily the case for diabetes and anxiety (16). However, despite numerous efforts, no definitive statement can yet be made on the complexity of the cause effect relationship between diabetes (and other metabolic disorders) and diverse types of psychosomatic and/or mood related disorders. In view of the situation, it seems reasonable to suggest that at present HpE could as well be plausible and feasible therapeutic alternative for diabetic patients with depression and anxiety as comorbidities. Therefore, efforts should be made to test the validity of our observations in patients suffering from such comorbidities.

Diabetes mellitus in rats, as well as in humans, is associated with alterations in brain monoaminergic system. Streptozotocin-induced diabetic rats have shown a significant inhibition of serotonergic functions in different brain regions (20). These neurochemical alterations were reversed when diabetic rats received insulin replacement therapy (21). Similar changes in serotonergic system are also linked to depression (22). Therefore, a functional link between diabetes and depression (23) may exist at the level of the brain monoamine system.

The observed anxiolytic activity of HpE in diabetic rats could be explained on the basis of augmented serotonin level in various parts of the brain by HpE treatment (24). Another interesting possibility is that the treatment with HpE could alleviate anxiety *via* modulation of GABAergic neurotransmission, since HpE has also been reported to inhibit reuptake of GABA (24). Facilitation of GABAergic neurotransmission by HpE is further supported by the fact that Hypericum has significant affinity for $GABA_A$ receptor in *in-vitro* receptor binding assay (25). Hypericum extract (26) as well as hyperforin has been reported to modulate CNS activities by virtue of stress alleviating property (27). Prolonged daily treatment with HpE or hyperforin has been reported to improve hyperglycemia and other metabolic disturbances in diabetic animals (6). Taken together, available information on the bioactivity profile of the HpE strongly suggest that this extract could be a real therapeutic option for diabetic patients with depression, anxiety and other functional disorders of the CNS.

In any case, recent observations made in our laboratories with HpE add experimental evidences to the conviction that controlling the stress related pathologies of the central nervous system could be a feasible approach for obtaining suitable pharmacotherapies for diverse multi-morbid conditions. Although epistemologically this conviction is quite familiar to many researchers and practitioners of mind body medicine, and more modern neuro-psycho-pharmacological findings continue to support this conviction, little concentrated efforts have till now been made to define herbal remedies in term of mind body medicine. Identifying and better characterizing the bioactive HpE constituents, and defining their modes and sites of actions and interactions, could be useful in bridging this gap.

CONCLUSION

Antidepressants and anxiolytic like effects of HpE are not altered in diabetic animals. These observations taken together with its recently observed beneficial effects in animal models of cognitive function, inflammation, stress and diabetes makes it a suitable candidate for developing a therapeutic principle potentially useful for helping diabetic patients with multiple comorbidities.

Conflict of interest

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

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REFERENCES

1. Muller W.E.: in St. John's Wort and its Active Principles in Depression and Anxiety, Muller W.E., Ed., p. 1, Birkhauser Verlag, Basel 2005.
2. Medina M.A., Martinez-Poveda B., Amores-Sanchez M.I., Quesada A.R.: Life Sci. 79, 105 (2006).
3. Kumar V., Singh P.N., Jaiswal A.K., Bhattacharya S.K.: Indian J. Exp. Biol. 37, 1171 (1999).
4. Kumar V., Jaiswal A.K., Singh P.N., Bhattacharya S.K.: Indian J. Exp. Biol. 38, 36 (2000).
5. Kumar V., Singh P.N., Bhattacharya S.K.: in Neuropsychopharmacological studies on Indian *Hypericum perforatum* Linn. In: Medicinal and Aromatic Plants – Industrial Profile, Volume Genus Hypericum, Ernst E., Ed. p. 179, Taylor & Francis, London, simultaneously published by Taylor & Francis Inc., New York 2003.
6. Husain G.M., Singh P.N., Kumar, V.: Drug Discov. Ther. 3, 215 (2009).
7. Husain G.M., Singh P.N., Kumar V.: Drug Discov. Ther. 3, 88 (2009).
8. Masiello P., Broca C., Gross R., Roye M., Manteghetti M., Hillaire-Buys D., Novelli M., Ribes G.: Diabetes 47, 224 (1998).
9. Ramanathan M., Jaiswal A.K., Bhattacharya S.K.: Psychopharmacology (Berl) 135, 361 (1998).
10. Massol J., Martin P., Puech A.J.: Diabetes 38, 1161 (1989).
11. Massol J., Martin P., Belon J.P., Puech A.J., Soubrie P.: Psychoneuroendocrinology 14, 145 (1989).
12. Massol J., Martin P., Chatelain F., Soubrie P., Puech A.J.: Pharmacol. Biochem. Behav. 31, 807 (1988).
13. Kamei J., Miyata S., Morita K., Saitoh A., Takeda H.: Pharmacol. Biochem. Behav. 75, 247 (2003).
14. Pouwer F.: Nat. Rev. Endocrinol. 5, 665 (2009).
15. Cohen B.E., Panguluri P., Na B., Whooley M.A.: Psychiatry Res. 175, 133 (2010).
16. Bouwman V., Adriaanse M.C., Van't Riet E., Snoek F.J., Dekker J.M., Nijpels G.: PLoS One 5, e9971 (2010).
17. Lin E.H.B., Heckbert S.R., Rutter C.M., Katon W.J., Ciechanowski P., Ludman E.J., Oliver M. et al.: Ann. Fam. Med. 7, 414 (2009).
18. Katon W.J.: Am. J. Med. 121, S8 (2008).
19. Golden S.H., Lazo M., Carnethon M., Bertoni A.G., Schreiner P.J., Diez Roux A.V., Lee H.B., Lyketsos C.: JAMA 299, 2751 (2008).
20. Sandrini M., Vitale G., Vergoni A.V., Ottani A. Bertolini A.: Life Sci. 60, 1397 (1997).
21. Chu P.C., Lin M.T., Shian L.R., Leu S.Y.: Diabetes 35, 481 (1986).
22. Price L.H., Charney D.S., Delgado P.L., Heninger G.R.: Am. J. Psychiatry 148, 1518 (1991).
23. Rodin G.M.: Am. J. Psychiatry 28, 219 (1983).
24. Kehr J., Ogren S.O., Yoshitake T.: in St. John's Wort and its Active Principles in Depression and Anxiety, Muller W.E. Ed., p. 47, Birkhauser Verlag, Basel 2005.
25. Gobbi M., Moia M., Pirona L., Morizzoni P., Mennini T.: Pharmacopsychiatry 34, S45 (2001).
26. Husain G.M., Chatterjee S.S., Singh P.N., Kumar V.: Pharmacologyonline 1, 432 (2009).
27. Kumar N., Husain G.M., Singh P.N., Kumar V.: Drug Discov. Ther. 3, 162 (2009).

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