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Ficus hispida modulates oxidative-inflammatory damage in a murine model of diabetic encephalopathy

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Abstract

Diabetes mellitus is a metabolic disorder characterized by persistent hyperglycemia, and insufficiency of insulin secretion and/or insulin resistance. Mounting evidence in both experimental and clinical studies suggests that oxidative stress plays a key role in the pathogenesis of diabetes mellitus and its complications. The objective of the present study was to investigate the effect of methanolic leaf extract of *Ficus hispida* (FHLE) on antioxidants, lipid peroxidation and inflammatory markers like tumor necrosis factor-alpha (TNF-α) activity in the brain of diabetic encephalopathy (DE) rats. DE rats displayed declined levels of endogenous antioxidants [superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH)], as well as elevated levels of malondialdehyde (MDA), nitric oxide (NO) and TNF-α. However, treatment with FHLE significantly precluded these alterations in the DE rats, demonstrating that FHLE can alter the oxidant stress and consequently improve the brain homeostasis.

Keywords: Diabetes mellitus, *Ficus hispida*, encephalopathy, oxidative stress.

Introduction

Recent estimates show that around 200 million people suffer from diabetes mellitus, making it the most common severe metabolic disorder worldwide [1]. In fact, the mechanism causing brain damage in diabetes mellitus remains still obscure, but it appears to be a multifactorial process which involves hyperglycemia, as well as inflammation and vascular disturbances, such as reduced cerebral blood flow [2] and alterations in cellular calcium homeostasis [3]. Studies propose that, through hyperglycemia, hyperlipidemia, hypertension and possible iron

dyshomeostasis, diabetes provokes oxidative stress that causes damage to multiple organs, leading to various complications including diabetic encephalopathy [4].

Ample studies advocate that multicomponent botanical extracts could alleviate these diverse alterations which lead to diabetic brain damage [5, 6]. Our previous studies substantiate the antioxidative and other beneficial effects of *Ficus hispida* leaf extract on the heart and liver [7, 8]. *Ficus hispida* Linn. is well known for its exploitation as an indigenous remedy in many liver ailments. Besides, Ghosh et al, [9] reported that *Ficus hispida* possess significant hypoglycemic activity and the beneficial effect of FHLE on brain was also reported [19, 20]. In this milieu, we hypothesized that FHLE could effectively mitigate hyperglycemia and oxidative stress, thereby ameliorating diabetic encephalopathy. To the best of our knowledge, this is the first report to demonstrate the beneficial effect of FHLE on the oxidative-inflammatory parameters in the brain.

Materials and Methods

Drugs and chemicals

All the drugs and biochemicals used in this experiment were purchased from Sigma Chemical Company Inc., St Louis, Mo, USA. The chemicals were of analytical grade. A glucose oxidase peroxidase diagnostic enzyme kit was purchased from Span Diagnostic Chemicals, India.

Collection and authentication of plant

The leaves of *Ficus hispida* Linn. (Moraceae) were collected from the herbal garden of Anna Siddha Hospital and Research Centre, Chennai, India. A voucher specimen (PARC/2007/Vel's/28) was deposited in the Plant Anatomy Research Centre, Pharmacognosy Institute, Chennai, India and was authenticated by Dr.Jayaraman.

Preparation of extract

The dried and powdered leaves were defatted with petroleum ether (B.P. 60–80°C) and then extracted with methanol in a Soxhlet extractor. On evaporation of methanol from the methanol extract *in vacuo*, a greenish coloured residue was obtained (yield 4.7% (w/w) with respect to the dry starting material) and was stored in a desiccator and used for further studies. The phytochemical evaluation was done as reported in our previous studies [7].

Procurement of animals

The study was conducted on male Wistar albino rats (180-230 g). Animals were obtained from the Animal House, Vel's College of Pharmacy, The Tamilnadu Dr. M.G.R. Medical University, Chennai, India. Animals were fed with commercially available standard rat pelleted feed (M/s Pranav Agro Industries Ltd., India) under the trade name Amrut rat/mice feed and water was provided *ad libitum*. The animals were deprived of food for 24 h before experimentation but allowed free access to tap water. The rats were housed under conditions of controlled temperature (23±2°C) and were acclimatized to 12-h light: 12-h dark cycles. Experimental animals were used after obtaining prior permission and handled according to the University and institutional legislation as regulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Induction of experimental diabetes

A freshly prepared solution of streptozotocin (60 mg/kg) in 0.1 M citrate buffer, pH 4.5 was injected intraperitoneally in a volume of 1 ml/kg. Blood samples were collected from tail vein 48 h after STZ administration. The rats with blood glucose more than 250 mg/dl were considered as diabetics and were further considered for study.

Study Design

The rats were allowed to acclimatize to the laboratory environment for 7 days before the start of the experiment. Rats were randomly selected and divided in five groups of 6–8 animals each. Group I consisted of non-diabetic control animals, group II was the diabetic controls, group IV consisted of diabetic animals treated with FHLE (400 mg/kg/day; p.o.), and group V comprised of diabetic animals treated with insulin (10 IU/kg/day, s.c.) group III comprised of non-diabetic control animals being administered with FHLE (400 mg/kg, p.o.) alone. FHLE was suspended in saline (containing 5% Tween-80) and was administered by oral gavage. Starting from the third day of experiment, group I and group II rats received vehicle of FHLE for 6 weeks.

Biochemical estimations

The animals were submitted to euthanasia being previously anesthetized with ether and the brains were removed and placed in a solution of 10mM Tris–HCl, pH 7.4, on ice. The brains were homogenized in a glass potter in Tris–HCl solution. Aliquots of the resulting brain homogenates were stored at −8°C until utilization. The activities of reduced glutathione, catalase, superoxide dismutase, NO and TNF-α as well as the concentrations of the malondialdehyde and the protein in the supernatant were determined by commercially available kits as per the manufacturer's instructions. Lipid peroxidation was assessed by measuring the concentration of malondialdehyde.

Statistical analysis

The results were expressed as mean ± standard deviation (S.D.) for 6-8 animals in each group. Differences between groups were assessed by one-way analysis of variance (ANOVA) using the SPSS 13.0 software package for Windows. Post hoc testing was performed for inter-group comparisons using the least significance difference (LSD) test. $p < 0.05$ was considered as statistically significant.

Results and Discussion

Diabetes mellitus is associated with glucose dysmetabolism which is paralleled by neurophysiological and structural changes in the brain [10]. Although, the underlying mechanisms involved in diabetes-induced CNS complications are coming under greater scrutiny, cellular and molecular pathophysiology of diabetic encephalopathy is not well delineated. Streptozotocin (STZ), a widely used experimental diabetogen is a glucose-like molecule with a nitrosourea moiety. Thus, it enters the β-cells through glucose transporters, thereby poisoning them resulting in diabetes. Conceptually, one unifying mechanism driving the pathophysiology of diabetic encephalopathy is believed to be oxidative stress, triggered by hyperglycemia and/or insulin resistance/deficiency. Mounting studies show that intervention of exogenous antioxidants might serve as a mainstay in the management of diabetic encephalopathy [11]. Even now, herbal extracts are being used by many ethnic healers in the treatment of diabetes and its complications.

Ficus hispida leaf is known to possess many antioxidant and anti-inflammatory compounds like hispidin, oleanolic acid, β-amyrin, β-sitosterol, etc. In view of this background, we assessed that FHLE might ameliorate the diabetic brain insult. In the present study, STZ treatment demonstrated significant increase in plasma glucose levels along with reduction in body weight (Table 1). On the contrary, FHLE administration at 400 mg/kg body weight reduced the blood glucose towards normal value which proves the anti-hyperglycemic effect of FHLE.

Table 1: The effect of FHLE and streptozotocin on body weight and fasting blood glucose levels in control and DE rats at the onset and the end of the study

Groups	Body weight (g)		Plasma glucose (mg/dL)	
	Onset of study	End of study	Onset of study	End of study
Control (I)	202.3 ± 17.2	236.3 ± 11.8	94 ± 6.1	96.3 ± 5.51
DE (II)	203.7 ± 16.1	164.7 ± 5.7[a,*]	92 ± 5.3	369 ± 14.5[a,*]
FHLE (III)	207.7 ± 13	241 ± 13.9	96 ± 5.9	91.7 ± 4.7
DE + FHLE (IV)	206 ± 13.1	198.3 ± 11.8[b,*]	90 ± 4.9	113.3 ± 5.1[b,*]
DE + INS (V)	210.3 ± 23.7	184.7 ± 16.7[c,*]	91 ± 5.1	121.3 ± 5.7[c,*]

Results are expressed as mean ± SD (n = 6-8 rats). Comparisons are made between [a]Group I and II; [b]Group II and IV; [c]Group II and V. [*]$p<0.05$.; FHLE – *Ficus hispida* leaf extract; DE – Diabetic encephalopathy; INS – Insulin.

The brain is predominantly vulnerable to oxidative assault because of its high oxygen utilization, its high content of oxidizable polyunsaturated fatty acids (PUFA), and the presence of redox-active metals (Cu, Fe). Compelling evidence in both experimental and clinical studies suggests that oxidative stress plays a vital role in the pathogenesis of diabetes mellitus and its complications. In harmony with the earlier studies, we found that STZ-induced experimental model for diabetes provides a relevant example of endogenous oxidative stress in brain, due to ensuing hyperglycemia [12]. Matching the assortment of oxidants, the endogenous antioxidant armamentarium comprises a widespread array of systems (antioxidant network). In our present study, we observed that diabetes attenuated the endogenous antioxidants in brain. Conversely, the levels of SOD, CAT, and GSH (Table 2) were effectively bolstered in the brain of FHLE treated DE rats which could be ascribed to the antioxidant and free-radical scavenging effect of the phytoconstituents. FHLE treated group was found to display a better activity than insulin treatment. Furthermore, insulin treatment failed to increase the SOD level to a significant level ($p<0.05$) when compared with the FHLE treated DE group.

Table 2: Effects of FHLE and streptozotocin on the levels of antioxidants and MDA in the brain tissues of control and DE rats

Groups	GSH	SOD	CAT	MDA
Control (I)	5.1 ± 0.2	8.7 ± 0.6	7.0 ± 0.4	3.1 ± 0.3
DE (II)	3.1 ± 0.3^{a,*}	6.0 ± 0.3^{a,*}	3.8 ± 0.3^{a,*}	5.0 ± 0.4^{a,*}
FHLE (III)	5.3 ± 0.2	8.9 ± 0.5	6.8 ± 0.4	2.9 ± 0.4
DE + FHLE (IV)	4.3 ± 0.2^{b,*}	7.6 ± 0.5^{b,*}	6.1 ± 0.5^{b,*}	3.6 ± 0.1^{b,*}
DE + INS (V)	4.0 ± 0.3^{c,*}	6.6 ± 0.4	5.1 ± 0.4^{c,*}	3.9 ± 0.2^{c,*}

Units: GSH (nmol/ mg wet tissue), SOD (Units/ mg protein), CAT (µmol H_2O_2 consumed min/ mg/ protein), MDA (nmol/ mg protein). Results are expressed as mean ± SD (n = 6-8 rats). Comparisons are made between [a]Group I and II; [b]Group II and IV; [c]Group II and V. [*]$p<0.05$.; FHLE – *Ficus hispida* leaf extract; DE – Diabetic encephalopathy; INS – Insulin; GSH – Reduced glutathione; MDA – Malondialdehyde; SOD – Superoxide dismutase; CAT – Catalase.

NO is a vital intracellular messenger molecule involved in many physiologic processes such as regulation of blood flow and neurotransmission as well as in pathological processes. The margin between the physiologic and pathologic activities of NO is still a matter of debate, but it is well known that both its concentration and production site are critical in determining whether it acts as a signaling or a neurotoxic molecule [13]. Peroxynitrite is a toxic compound formed during the inactivation of nitric oxide (NO) by the superoxide anion. Molecular biology studies suggest a sturdy link between implication of increased formation of peroxynitrite, superoxide anion and nitrotyrosine in high glucose level and their membranodestuctive effect on cellular integrity [14, 15]. Lipid peroxidation is especially a consequence of oxidative assault of the membrane phospholipids. Hydroxyl radicals produced in diabetic status react with transition metals like iron or copper to form stable aldehydes such as malondialdehydes that will cause deleterious effect on brain. In our study, the escalated levels of NO (Figure 1) and MDA (Table 2) clearly implicate the nitroxidative stress and lipid peroxidation in DE rats. The anti-lipid peroxidative and free radical scavenging effects of the FHLE are also evident from the significant ($p<0.05$) alleviation of these abnormalities in the brain of FHLE treated DE rats.

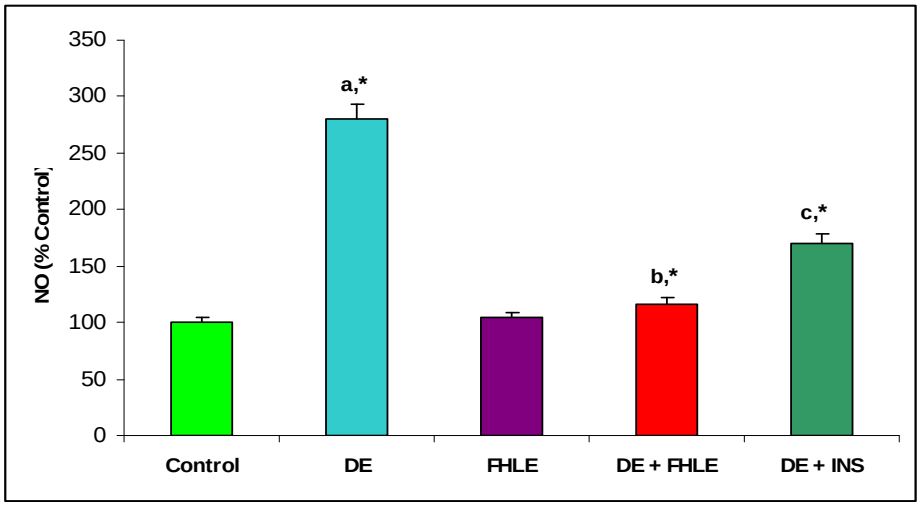

Fig 1: Effects of FHLE and streptozotocin on the nitric oxide (NO) levels in the brain of control and DE rats

Results are expressed as mean ± SD (n = 6-8 rats). Comparisons are made between [a]Group I and II; [b]Group II and IV; [c]Group II and V. [*]$p<0.05$.; FHLE – *Ficus hispida* leaf extract; DE – Diabetic encephalopathy; INS – Insulin.

Tumor necrosis factor (TNF-α) is a potent proinflammatory cytokine involved in many pathophysiological conditions including diabetic encephalopathy [16]. Igarashi et al, [17] proposed that there exists a causal relationship between hyperglycemia, PKC-β activation and increased inflammatory marker expression in diabetic rats. In harmony with that report, our study also showed increased TNF-α level (Figure 2) in DE rats. Intriguingly, a research study reported that hispidin attenuated STZ-induced diabetic microvasculopathy [18] which advocates that hispidin, a phytoconstituent present in FHLE could be a plausible candidate in ameliorating diabetic encephalopathy.

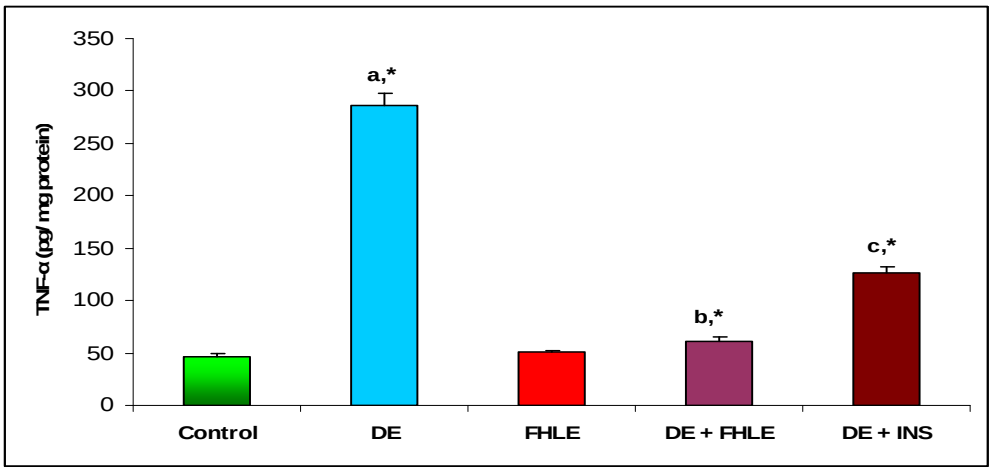

Fig 1: Effects of FHLE and streptozotocin on the tumor necrosis factor - alpha (TNF-α) levels in the brain of control and DE rats

Results are expressed as mean ± SD (n = 6-8 rats). Comparisons are made between [a]Group I and II; [b]Group II and IV; [c]Group II and V. [*]$p<0.05$.; FHLE – *Ficus hispida* leaf extract; DE – Diabetic encephalopathy; INS – Insulin.

Conclusion

In summary, the obtained results suggested that in diabetes mellitus, brain was more vulnerable to oxidative insult and inflammation. The cytoprotective potential of *Ficus hispida* leaf extract (FHLE) may be anticipated to have biological significance in eliminating reactive oxidative-nitrosative species that may otherwise affect normal brain homeostasis. Anti-hyperglycemic activity in FHLE along with neuroprotective effect against streptozotocin challenge provides a scientific rationale for the use of *Ficus hispida* as an anti-diabetic plant. These results may contribute to a better understanding of the neuroprotective role of FHLE, emphasizing the influence of the phytoconstituents including hispidin, oleanolic acid, β-amyrin, β-sitosterol and other antioxidants in the diet for human health, possibly preventing brain disorders associated with diabetes mellitus.

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References

[1] American Diabetes Association, *Diab Care*, **2007**, 17, 1514–1522.
[2] SM Manschot; JG Biessels; NE Cameron; MA Cotter; A Kamal; LJ Kappelle; WH Gispen. *Brain Res*., **2003**, 966, 274–282.
[3] NE Cameron; SEM Eaton; MA Cotter; S Tesfaye. *Diabetologia*, **2001**, 44, 1450–1458.
[4] W Wei; Q Liu; Y Tan; L Liu; X Li; L Cai, *Hemoglobin*, **2009,** 33, 5, 370-377.
[5] L Pari; M Latha, *BMC Complement Altern Med.*, **2004,** 2, 4, 16.
[6] MH Jang; H Kim; MC Shin; BV Lim; TH Lee; SB Jung; CJ Kim; EH Kim, *Jpn J Pharmacol.*, **2002,** 90, 2, 189-192.
[7] TS Shanmugarajan; M Arunsundar; I Somasundaram; D Sivaraman; E Krishnakumar; V Ravichandran, *J Pharmacol Toxicol*., **2008**, 3, 5, 363-372.
[8] TS Shanmugarajan, N Prithwish, I Somasundaram, M Arunsundar, M Niladri, JP Lavande, V Ravichandiran. *Toxicol Mech Methods*, **2008,** 18, 8, 653-60.
[9] R Ghosh; Kh Sharatchandra; S Rita; IS Thokchom, *Indian J Pharmacol*., **2004**, 36, 4, 222-225.
[10] GJ Biessels; IJ Deary; CM Ryan, *Lancet Neurol.*, **2008**, 7, 184–190.
[11] A Zarros; C Liapi; P Galanopoulou; K Marinou; Z Mellios; N Skandali; H Al-Humadi; F Anifantaki; E Gkrouzman; S Tsakiris, *Metab Brain Dis*., **2009**, 24, 337–348.
[12] AC Maritim; RA Sanders; JB Watkins III, *J Biochem Mol Toxicol.*, **2003**, 17, 1, 24-38.
[13] GC Brown, *Biochim Biophys Acta*, **1999**, 1411, 2-3, 351-369.
[14] A Soneja; M Drews; T Malinski, *Pharm Rep*, **2005**, 57 suppl 108-119.
[15] K Stadler; MG Bonini; S Dallas; J Jiang; R Radi; RP Mason; MB Kadiiska, *Free Radic Biol Med*., **2008**, 45, 6, 866–874.
[16] AA Sima; W Zhang; CW Kreipke; JA Rafols; WH Hoffman, *Rev Diabet Stud.*, **2009**, 6, 1, 37-42.

[17] M Igarashi; H Wakasaki; N Takahara; H Ishii; ZY Jiang; T Yamauchi; K Kuboki; M Meier; CJ Rhodes; GL King, *J. Clin. Invest.*, **1999**, 103, 185–195.
[18] Yuan SY, Ustinova EE, Wu MH, Tinsley JH, Xu W, Korompai FL, Taulman AC. *Circ Res.*, **2000**, 1, 87, 5, 412-417.
[19] D Sivaraman; P Muralidaran, *Drug Invention Today*, **2009**, 1, 1, 23-27.
[20] D Sivaraman; P Muralidaran, *J Herbal Med Toxicol*., **2009**, 3, 2, 147-150.